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GBA 2016476
GB 1421448
GB 1227211
GB 1161419
GB 1485502
GB 1446965
Enzyme Nomenclature, Recommendations (1978) of the Nomenclature Commitee of the International Union of Biochemistry; Academic Press (1979)—enzymes designated E.C.3.2.1.— which hydrolyse polysacchorides
(58) Field of search
C3H
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(54) **Improvement in and relating to an enzyme for decomposition of a high molecular carbohydrate, the isolated high molecular carbohydrate, a method for selection of a microorganism producing such enzyme and a method for production of such enzyme.**

(57) The enzyme, which is able to decompose a high molecular carbohydrate, abbreviated SPS (soluble polysaccaride), is designated SPS-ase. The SPS-ase is able to decompose SPS into decomposition products which attach themselves to protein in an aqueous medium to a lesser extent than the SPS prior to decomposition. The method for selection of SPS-ase producing microorganisms is based on the fact that the main carbon source of a growth medium is SPS and on a qualitative SPS-agar plate test. A method for production of an SPS-ase by means of a deposited strain of *Asp. aculeatus* is described. The SPS-ase also has utility in the fruit and vegetable industry and for production of juice and wine.

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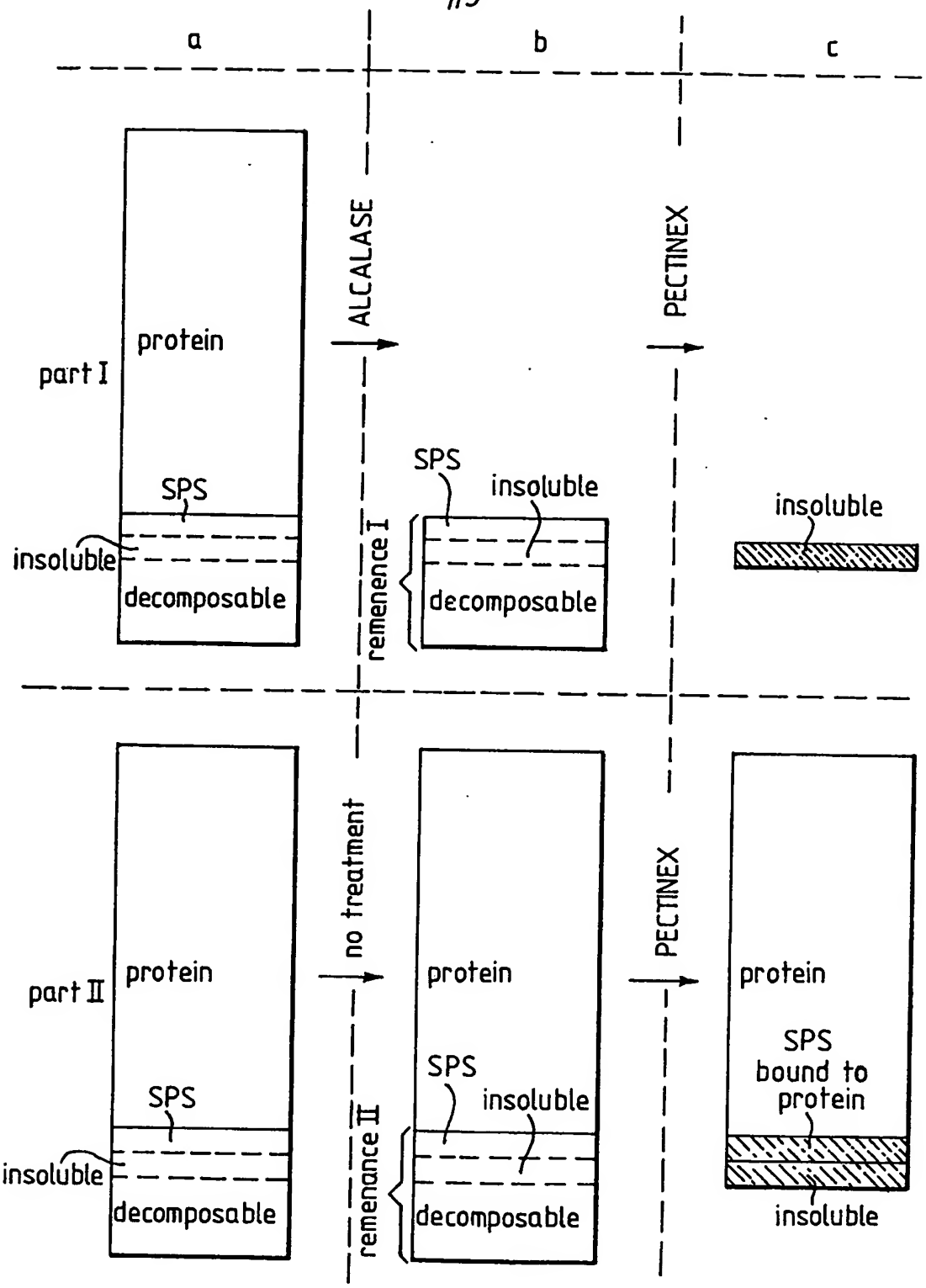

a
b
c
ALCALASE
PECTINEX
part I
protein
SPS
insoluble
decomposable
remenence I
SPS
insoluble
decomposable
insoluble
part II
protein
SPS
insoluble
decomposable
no treatment
remenance II
protein
SPS
insoluble
decomposable
PECTINEX
protein
SPS
bound to
protein
insoluble

Fig. 1.

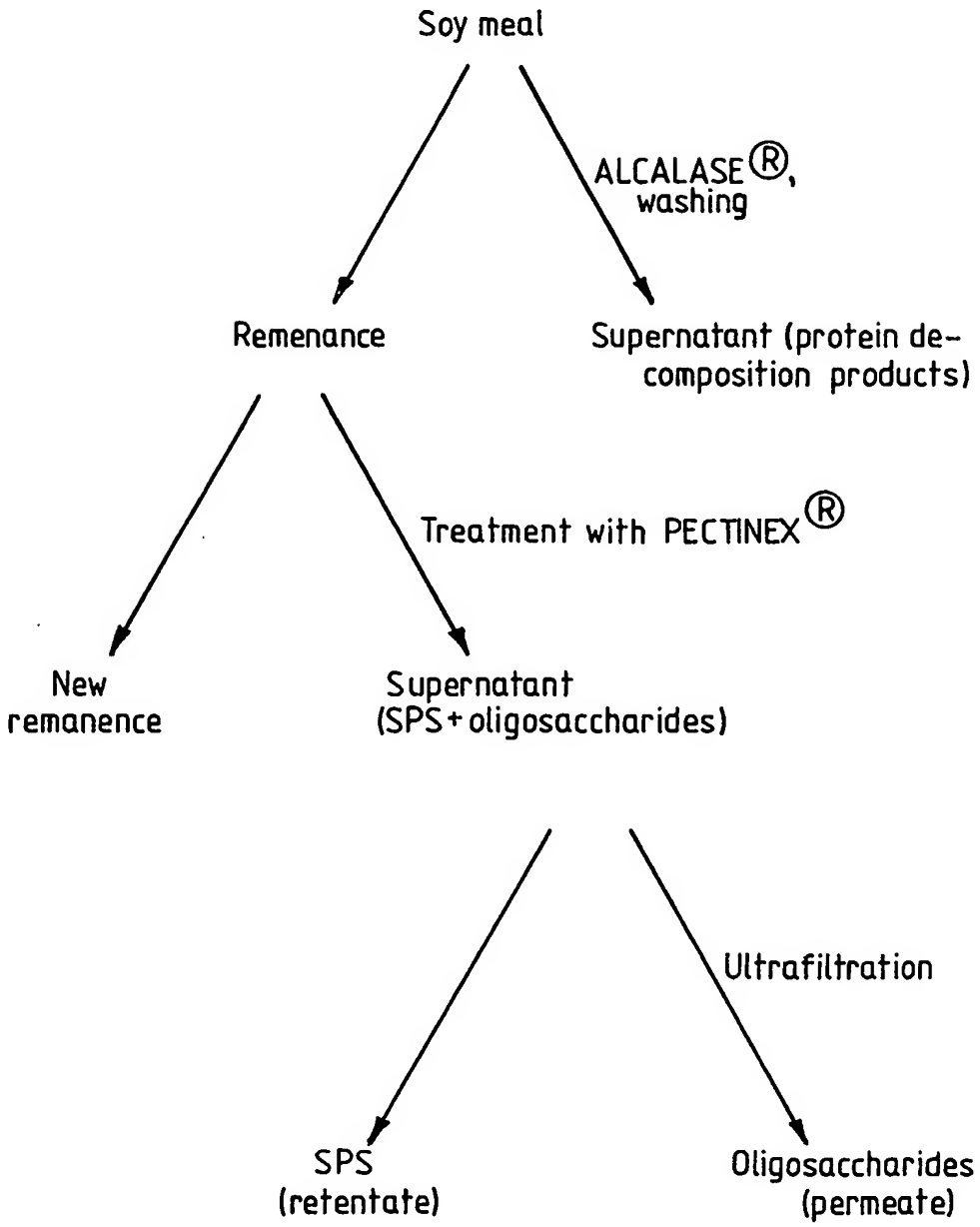

Soy meal
ALCALASE®, washing
Remenance
Supernatant (protein de-composition products)
Treatment with PECTINEX®
New remanence
Supernatant (SPS + oligosaccharides)
Ultrafiltration
SPS (retentate)
Oligosaccharides (permeate)

Fig. 2.

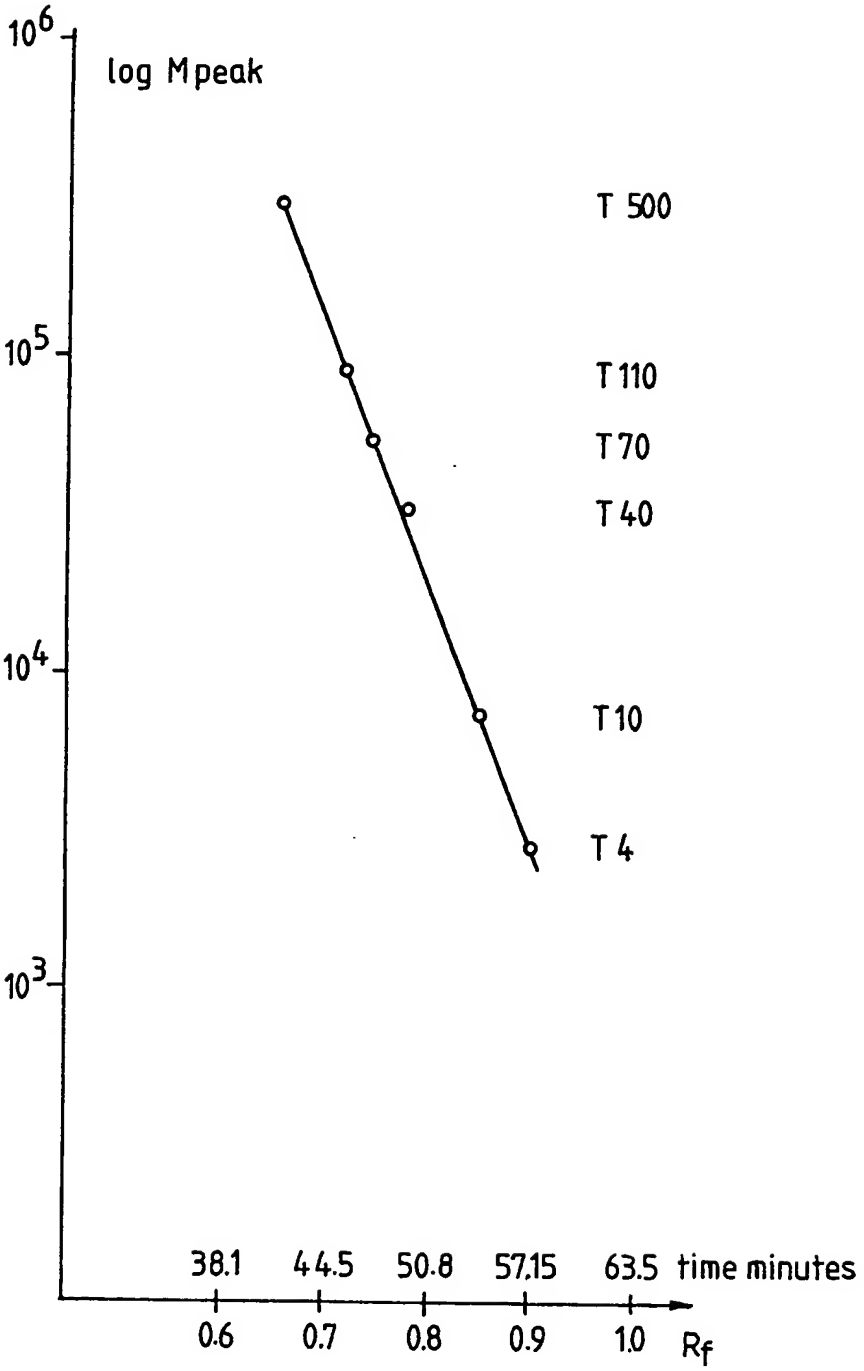

10^6
log Mpeak
10^5
10^4
10^3
T 500
T 110
T 70
T 40
T 10
T 4
38.1
44.5
50.8
57.15
63.5
time minutes
0.6
0.7
0.8
0.9
1.0
Rf

Fig. 3.

Fig. 4.

Fig. 5.

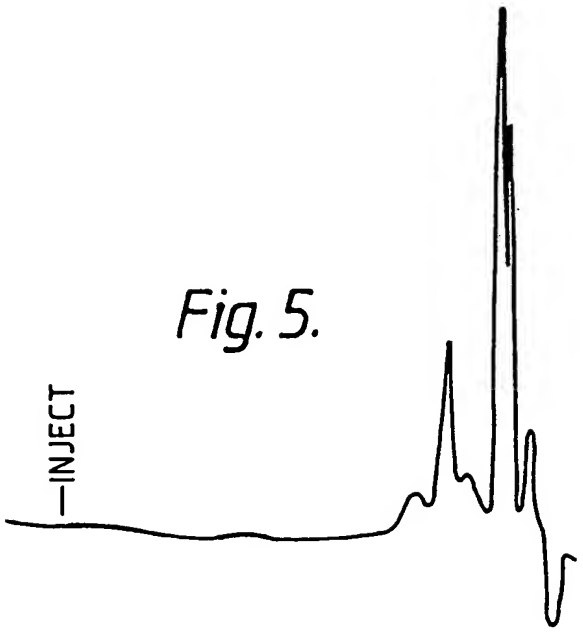


Fig. 6.

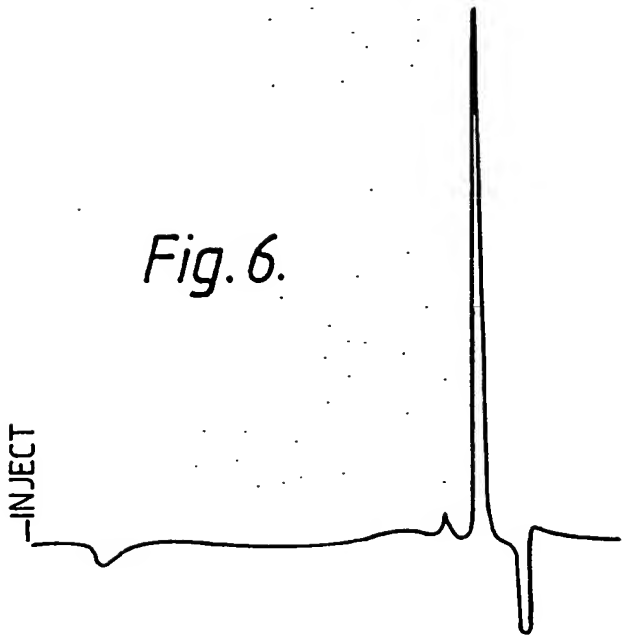


Fig.7.

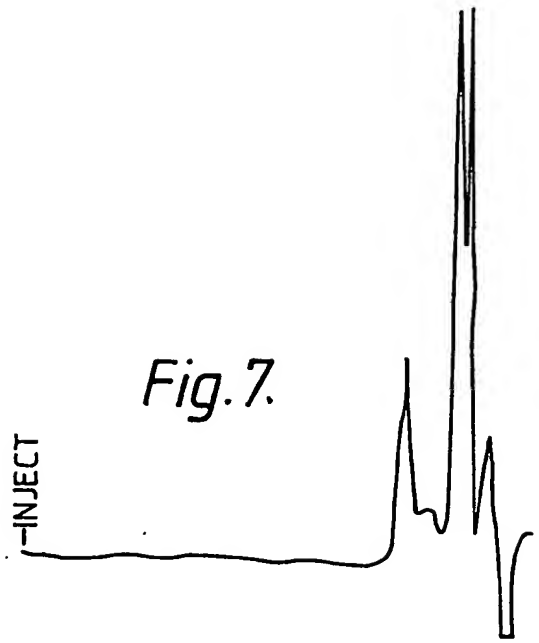


Fig.8.

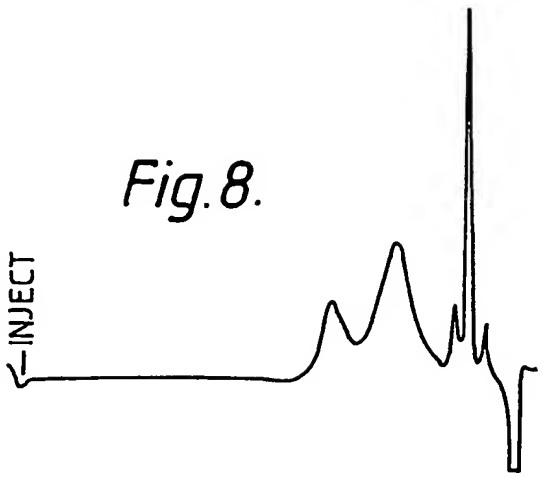
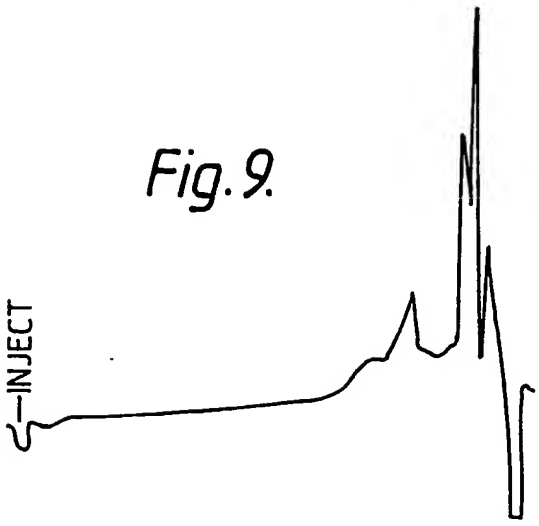


Fig.9.



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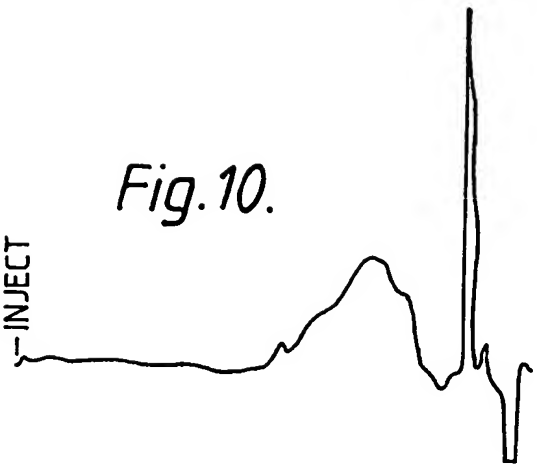

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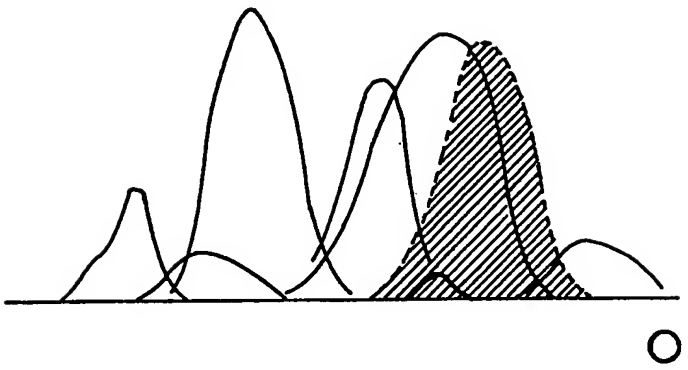


Fig. 11.

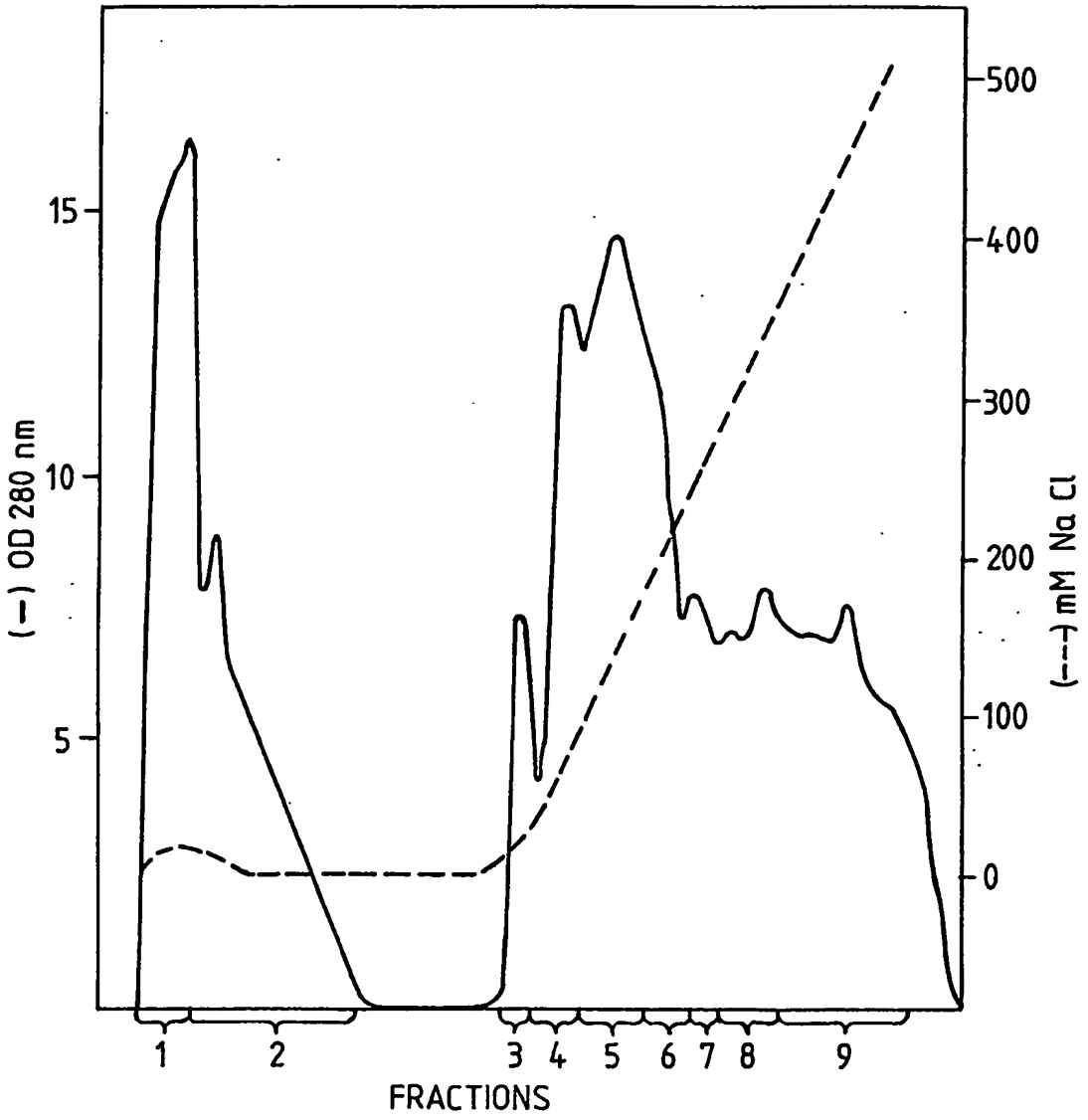

15
10
5
(—) OD 280 nm
500
400
300
200
100
0
(---) mM Na Cl
1
2
3
4
5
6
7
8
9
FRACTIONS

Fig. 12.

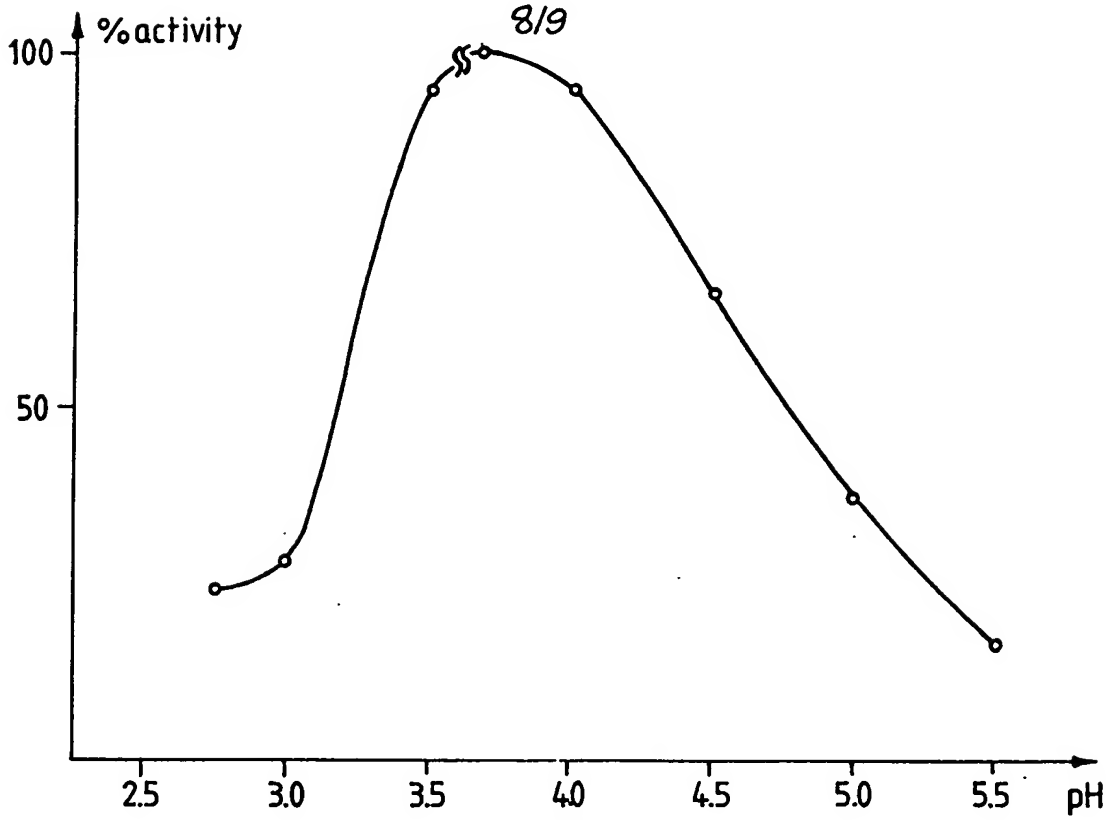

%activity
100
50
2.5
3.0
3.5
4.0
4.5
5.0
5.5
pH

Fig.13.

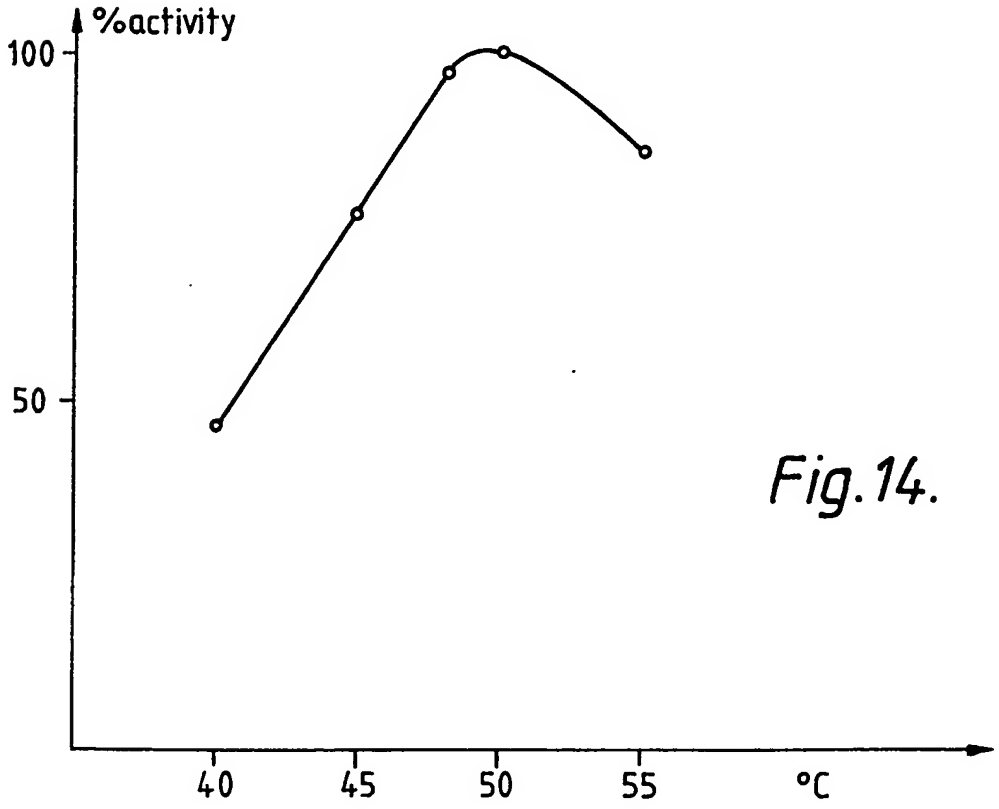

%activity
100
50
40
45
50
55
°C

Fig.14.

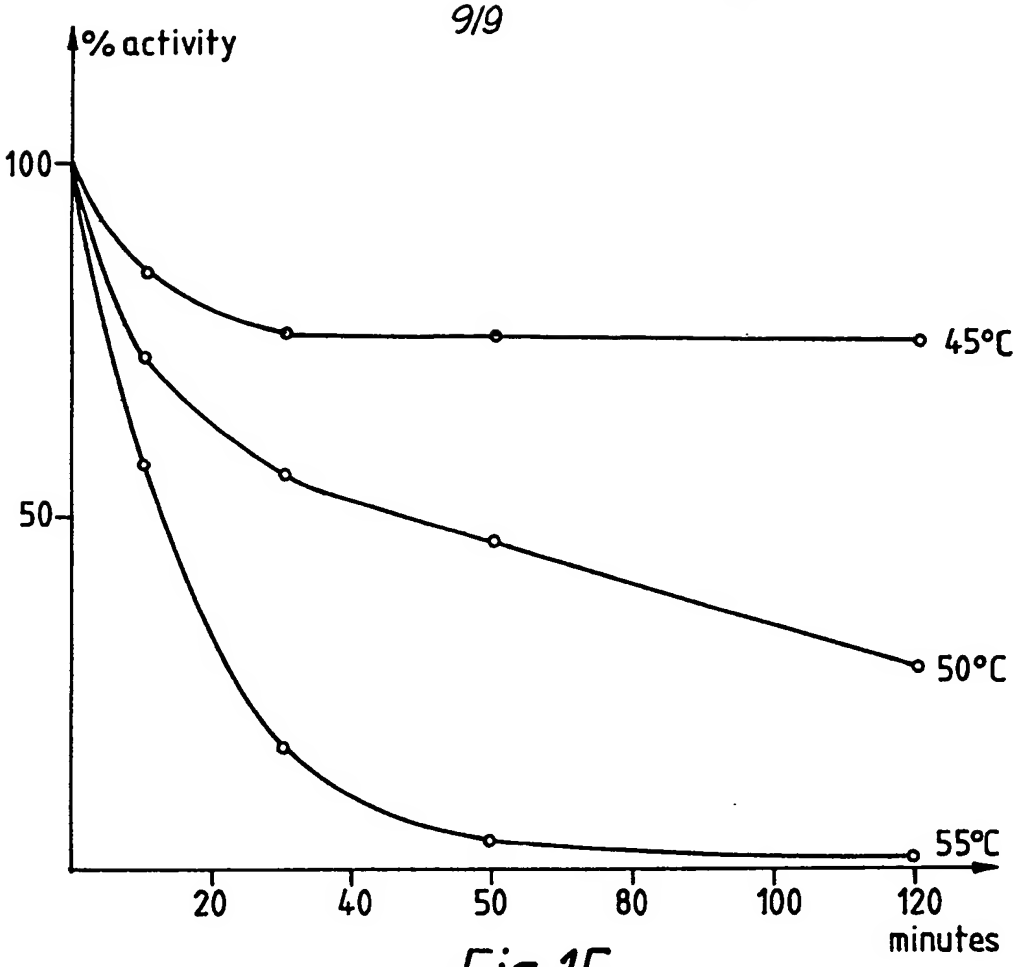

% activity
100
50
45°C
50°C
55°C
20
40
50
80
100
120
minutes

Fig.15.

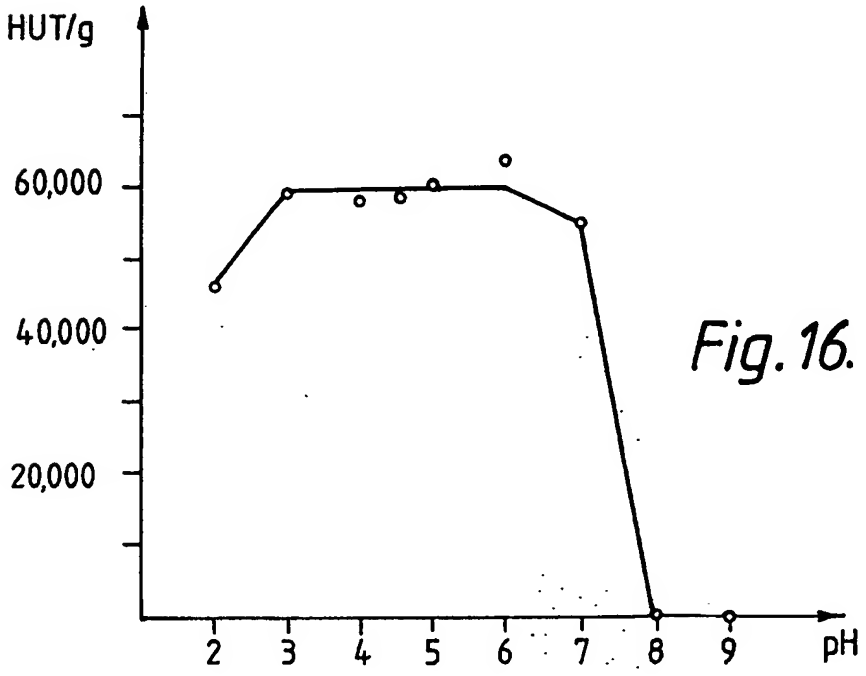

HUT/g
60,000
40,000
20,000
2
3
4
5
6
7
8
9
pH

Fig.16.

SPECIFICATION

Improvements in and relating to an enzyme for decomposition of a high molecular carbohydrate, the isolated high molecular carbohydrate, a method for selection of a microorganism producing such enzyme and a method for production of such enzyme

A method for production of a purified vegetable protein product (pvp) by enzymatic removal of the remanence, without dissolution and reprecipitation of the protein, is described in BE patent No. 822.769. The purity of the pvp obtainable by the known method is not satisfactory and therefore open to improvement. In the examples a purity of the pvp of about 85% was demonstrated. Even if it is possible to obtain a pvp of about 90% purity according to the known method, this is only obtainable with certain pretreated starting materials, e.g. soy protein concentrate. It would be desirable to be able to obtain a purity of the pvp of around or above 90% with a much broader spectrum of starting materials, especially dehulled and defatted soy meal.

The invention is based upon the surprising discovery that a certain part of the remanence decomposition product, as it appears during the enzymatic treatment indicated above, i.e. a water soluble, high molecular carbohydrate, attaches itself to part of the vegetable protein, as will be explained later in detail. This, of course, results in a lower purity of the protein. Also, it has been found that this high molecular carbohydrate has an ability to attach itself to proteins of animal origin.

Thus, an object of the invention is to provide an enzyme for decomposition of the above indicated high molecular carbohydrate, which will open up the possibility for production of a pvp with improved purity, and a method for production of such an enzyme.

The basis for the invention can be described in the following manner, reference being made to Fig. 1, in which only material existing as undissolved solids is indicated, whereas all supernantants are left out. A charge of soy meal was divided in two equal parts, part I and part II (column a in Fig. 1). Part I was decomposed proteolytically at a pH value of about 8 by means of ALCALASE (a proteolytic enzyme produced by means of B. licheniformis and marketed by NOVO INDUSTRI A/S, 2880 Bagsvaerd, Denmark), and then further washed at around pH 8 in order to eliminate the entire amount of protein, and the remanence was separated from the supernatant and washed (vide part I, column a and b, Fig. 1). In this way a pure remanence (designated remanence I) was isolated (column b in Fig. 1). Part II of the soy meal was not treated; for the sake of brevity the remanence in part II is designated remanence II (column b in Fig. 1). Now, both remanence I and part II are decomposed by means of a commercial pectinase, e.g. PEXTINEX (a pectolytic enzyme produced by Schweizerische Ferment A/G, Basle, Switzerland) (vide column b and c in Fig. 1). Surprisingly it is found that the undissolved part of remanence I is much smaller than the undissolved part of remanence II, on the basis of nitrogen and dry matter mass balances, vide Fig. 1, where the hatched areas in column c correspond to insoluble non-protein materials in the above indicated stage. Furthermore, if the supernatant from the pectinase treated remanence I is brought together with a soy protein suspension at pH 4.5, a polysaccharide in the supernatant is bound to the soy protein. This polysaccharide in the supernatant from remanence I, which is a part of the remanence decomposition product and which is clearly soluble in water in the absence of soy protein, but bound to soy protein at or around the isoelectric point of soy protein, if soy protein is present, is designated SPS (Soluble Polysaccharide), vide Fig. 1. The SPS has a molecular weight distribution between 5×10^6 and 4.9×10^4. The production of isolated SPS appears from the flow sheet shown on Fig. 2, which also encompasses some of the processes depicted in Fig. 1. Thus, the problem is to find an enzyme which is able to decompose the SPS in such a manner that the SPS decomposition products do not bind soy protein or do bind soy protein to a much lesser extent than SPS binds soy protein.

Although the disclosure specifically refers to soy protein, the invention is not restricted to soy protein, but encompasses all kinds of vegetable proteins, vide e.g. the proteins listed in BE patent No. 882.769, page 1.

Now, according to the invention it has been found that by screening for the ability to decompose soy SPS it is possible to select microorganisms which are able to produce a compounds which exhibits an enzymatic activity effectively decomposing soy SPS, in the following for the sake of brevity designated an SPS-ase.

Thus the invention in its first aspect comprises an SPS-ase, a carbohydrase in a usable form and capable of decomposing soy SPS under appropriate conditions into decomposition products which attach themselves to protein in an aqueous medium to a lesser extent than the soy SPS prior to decomposition would have attached itself to the same protein under corresponding conditions.

Furthermore it has been found that this SPS-ase capable of degrading soy SPS is able to degrade polysaccharides similar to SPS and originating from vegetables and fruits more completely than commercial pectinases and commercial cellulases.

The above indicated expression "in a usable form" is intended to exclude from the invention e.g. an SPS-ase containing preparation which contains toxic substances or which exhibits such low SPS-ase activity, which necessitates the use of more than 10% SPS-ase containing preparation, related to the weight of SPS in the substrate, in a reaction conducted for 24 hours and at 50°C, at the pH optimum of the SPS-ase in question, in order to obtain a decomposition of SPS of any practical importance.

By total or partial elimination of the SPS from the final vegetable protein the purity of the final vegetable protein necessarily is improved in comparison with the purity of the final vegetable protein obtainable according to the method known from BE patent No. 882.769, as this known vegetable protein product was contaminated with SPS.

At present it is not known if the particular SPS-ase described in the following derives its enzymatic activity from a single enzyme or from an enzyme complex comprising at least two enzymes. Some investigations seem to indicate that at least two enzymes are responsible for the SPS-ase degradation effect, whereby one of these enzymes is capable of carrying out only a slight decomposition of SPS, whereafter one or more enzymes are able to perform a more extensive degradation of the SPS. The applicant, however, does not want to be restricted by such hypothesis or similar hypotheses.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the SPS-ase is capable of decomposing soy SPS in an aqueous medium into decomposition products which attach themselves to vegetable protein in the aqueous medium to a lesser extent than the soy SPS prior to decomposition would have attached itself to the same vegetable protein in the aqueous medium.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the SPS-ase is capable of decomposing soy SPS in an aqueous medium with a pH value not deviating more than 1.5 from 4.5 into decomposition products which attach themselves to soy protein in the aqueous medium to a lesser extent than the soy SPS prior to decomposition would have attached itself to the soy protein in the aqueous medium.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the decomposition products of soy SPS after completed degradation attach themselves to the vegetable protein to an extent of less than 50%, particularly less than 20% than the soy SPS prior to decomposition would have attached itself to the vegetable protein in the aqueous medium.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the SPS-ase exhibits a positive SPS-ase test, when examined according to the qualitative and quantitative SPS-ase determination method described in this specification.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the SPS-ase was produced by means of a microorganism belonging to the genus Aspergillus, preferably belonging to the Aspergillus niger group.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the SPS-ase is derived from the enzymes producible by means of Asp. aculeatus CBS 101.43. The same SPS-ase can be produced by means of Asp. japonicus IFO 4408. It has been found that Asp. aculeatus CBS 101.43 also produces very potent remanases, cellulases, pectinases, and hemicellulases. Furthermore, it has been found that not each and every strain belonging to the species Asp. aculeatus or Asp. japonicus generates an SPS-ase needed for the invention. Thus, as it appears later in this specification (section 5), it has been demonstrated that the strain Asp. japonicus ATCC 20236 does not produce such amounts of an SPS-ase which can be detected by means of the enzymatic determination of SPS-ase described in this specification.

A preferred embodiment of the SPS-ase according to the invention is characterized by the fact that the SPS-ase is immunoelectrophoretically identical to the SPS-ase producible by means of Asp. aculeatus CBS 101.43 and identifiable by means of the immunoelectrophoretic overlay technique, vide section 6 and 7.

When an SPS-ase is produced microbially, it is formed in admixture with several accompanying substances, particularly other enzymes. If desired, the SPS-ase in question can be purified, e.g. by means of chromatographic separation methods, as will appear later in this specification (section 8).

In Agr.Biol.Chem 40 (1), 87—92, 1976 it is described that a strain of Asp. japonicus, ATCC 20236, produces an enzyme complex which is able to perform a partial degradation of an acidic polysaccharide in soy sauce, named APS, a fraction of which is designated APS—I. This acidic polysaccharide is not identical to SPS, which will be shown later in this specification in more detail in section 3. Thus, the HPLC gel filtration chromtograms of SPS and APS are clearly different, and furthermore, the gel filtration chromtograms of APS decomposed by means of the commercial pectinase Pectolyase and of SPS treated with the commercial pectinase Pectolyase are clearly different. Furthermore, it does not appear from the article that the acidic polysaccharide is bound to the soy protein and that the decomposed acidic polysaccharide is not bound to the soy protein or is bound to the soy protein to a much lesser extent than the undecomposed acidic polysaccharide. Also, it has been demonstrated that this strain does not form SPS-ase in such amounts which can be detected by means of the enzymatic determination of SPS-ase described in this specification. This created a prejudice against any strain of Asp. japonicus being a producer of an SPS-ase, but surprisingly according to the invention it has been found that some strains of Asp. japonicus are producers of an SPS-ase.

The invention comprises in its second aspect the isolated SPS, produced on the basis of vegetable raw protein as a starting material.

A preferred embodiment of the isolated SPS according to the invention is characterized by the fact that the vegetable raw protein is defatted soy meal. The production of this isolated SPS is described in the foregoing in relation to Figure 2.

The invention comprises in its third aspect a method for selection of an SPS-ase producing microorganism for production of the SPS-ase according to the invention, wherein the microorganism to be tested is grown on a fermentation medium, the main carbon source of which is SPS, whereafter a sample of the fermentation medium is analyzed for SPS-ase and the microorganism in question is selected as an SPS-ase producing microorganism, if the analysis for SPS-ase is positive.

The invention comprises in its fourth aspect a method for production of SPS-ase, wherein a strain selectable according to the above method of selection of an SPS-ase producing microorganism is cultivated in a nutrient medium. The cultivation can be carried out as a submerged fermentation or as a surface fermentation.

A preferred embodiment of the method according to the invention is characterized by the fact that the strain Asp. aculeatus CBS 101.43 or Asp. japonicus IFO 4408 is cultivated in a nutrient medium.

A preferred embodiment of the method according to the invention is characterized by the fact that cultivation is carried out as a submerged cultivation at a pH in the range of from 3 to 7, preferably from 4 to 6, at a temperature in the range of from 20 to 40°C, preferably from 25 to 35°C, and whereby the nutrient medium contains carbon and nitrogen sources and inorganic salts.

A preferred embodiment of the method according to the invention is characterized by the fact that the nutrient medium contains toasted soy meal.

A preferred embodiment of the method according to the invention is characterized by the fact that the soy meal is treated with a proteolytic enzyme before the use as a component of the substrate, preferably the proteolytic enzyme produced microbially by means of Bacillus licheniformis.

A preferred embodiment of the method according to the invention is characterized by the fact that a solution of pectin is added aseptically to the fermentation broth during the cultivation.

It has been found that Asp. aculeatus CBS 101.43 also produces very potent remanence solubilizing enzymes, cellulases, pectinases, and hemicellulases besides the SPS-ase, and that the enzyme complex produced by means of Asp. aculeatus CBS 101.43 is excellently suited as an agent for use in cell wall disintegration of vegetable materials. Thus, the enzyme complex producible from Asp. aculeatus CBS 101.43 can be used in the food processing industry for treatment of fruit and vegetable mashes and for clarifying and viscosity reducing purposes in the processing of juice and wine with excellent results; also, it can be used as a dewatering agent (i.e. an agent for decomposition of polysaccharides and hence for liberation of the water bound within the polymeric structure of the polysaccharides) in the processing of vegetables.

Thus, in its fifth aspect, the invention comprises a use of an SPS-ase or a method for decomposition of polysaccharides, preferably plant cell wall polysaccharides, by means of a carbohydrase wherein the SPS-ase preparation according to the invention in an aqueous medium is contacted with a substrate for said SPS-ase preparation.

Thus, according to the invention it has been found that SPS-ase preparations are valuable enzyme preparations for partial or total liquefaction or decomposition of several materials, preferably vegetable materials, e.g. fruits, and plant wastes, containing protein, cellulose, hemicellulose, (e.g. glucans, xylan, galactans, and araban) gums, pectin, lipids, inulin, polyphenols, starch, and alginates, and for purposes related thereto, vide the table shown later in this specification. As examples of such related purposes may be mentioned all purposes for which commercial pectinases and cellulases are used today. Several examples will be given later in this specification.

In relation to the extraction (isolation) process described in for instance Example 2 it is noted that the SPS-ase preparation is essentially proteinase free, due to the fact that the wanted end product, i.e. the protein, otherwise would be degraded. Likewise, if it is wanted to extract (isolate) other biological materials than protein from a raw biological material, the SPS-ase preparation used should be essentially free of any enzyme degrading this other biological material. Such modified SPS-ase preparations can be produced by selective inactivation of the undesired enzyme, by fractionation, or by other methods known per se.

Thus, a preferred embodiment of the use according to the invention is characterized by the fact that the decomposition is accompanied by the isolation or extraction of a biological material other than soy protein and related vegetable proteins from a raw biological material whereby the SPS-ase preparation is essentially free of any enzyme which is able to degrade said biological material.

Thus, according to the invention it has been found that the modified SPS-ase preparations (modified in the sense that they are essentially free of enzymes capable of degrading the biological material to be extracted or isolated) are valuable enzyme preparations for extraction (isolation) of specified biological materials, e.g. starch, lipids, flavours, colours, and juices, from raw biological materials. Several examples will be given later in this specification.

A preferred embodiment of the use according to the invention is characterized by the fact that one or more of the reaction products (no matter whether they are wanted end products or waste products) are treated further simultaneously with or after the enzyme treatment. Hereby a more flexible and adaptable use is provided.

A preferred embodiment of the use according to the invention is characterized by the fact that the further treatment is an alcoholic fermentation in case one of the reaction products is a fermentable sugar. Hereby a simple and cheap method for production of alcohol is provided.

In order to clarify the nature of the invention, reference is made to the following sections 1 to 10, all describing details related to the invention:

1. Production of SPS.
2. Characterization of SPS, especially molecular weight distribution thereof.
3. Documentation for the fact that SPS and APS are different compounds.
4. Screening for SPS-ase producing microorganisms.
5. Characterization of some SPS-ase forming microorganisms.
6. General description of overlay technique associated with immunoelectrophoresis.
7. Immunoelectrophoretic characterization of SPS-ase with polyspecific antibody and overlay.
8. Purification of an SPS-ase preparation.
9. pH-activity dependency, temperature activity dependency, and stability of an SPS-ase.
10. Enzymatic activity determinations.

SECTION 1

PRODUCTION OF SPS

As previously mentioned the starting material for production of SPS may be soy remanence. Therefore, in the first place, the production of soy remanence is described.

Soy remanence is the protein free carbohydrate fraction (which in practice may contain minor amounts of lignin and minerals) in defatted and dehulled soy meal, which carbohydrate fraction is insoluble in an aqueous medium at pH 4.5, and it can be produced in the following manner, reference also being made to flow sheet 1.

Defatted soy meal (Sojamel 13 from Aarhus Oliefabrik A/S) is suspended in deionized water of 50°C in a weight proportion soy meal:water = 1:5 in a tank with pH-stat and temperature control. pH is adjusted to 8.0 with 4N NaOH (I). Now a pH-stat hydrolysis is performed with ALCALASE 0.6 L (a proteolytic enzyme on the basis of B. licheniformis with an activity of 0.6 Anson units/g, whereby the activity is determined according to the Anson method, as described in NOVO ENZYME INFORMATION IB No. 058 e-GB), whereby the ratio enzyme/substrate equals 4% of the amount of protein in the soy meal (II). After a hydrolysis of 1 hour the sludge is separated by centrifugation (III) and washing (IV) whereby this operation is performed twice (V, VI, VII). The thus treated sludge is hydrolyzed once more for 1 hour with ALCALASE 0.6 L (VIII, IX) similarly as indicated before. Then the sludge is separated by centrifugation (X) and washed twice (XI, XII, XIII, XIV), whereby the final washed sludge (6) is spray-dried (XV). The thus produced spray-dried powder is the soy remanence serving as a raw material for the production of SPS.

SPS is the water soluble polysaccharide fraction which is formed by conventional treatment of the above indicated soy remanence with pectinase. The SPS is produced in the following manner by means of the below indicated 14 reaction steps, reference also being made to flow sheet 3.

1. The dry matter content in the above indicated soy remanence is determined and the soy remanence is diluted with water to 2% dry matter and kept in suspension at 50°C in a tank with temperature control.
2. The pH value is adjusted to 4.50 with 6N NaOH.
3. Pectinex Super conc. L is added in an amount of 200 g/kg dry matter (a commercial pectinase from Schweizerische Ferment AG, Basle, Switzerland with a pectinase activity of 750,000 MOU, as determined according to the leaflet "Determination of the Pectinase units on Apple Juice (MOU)" of 12.6.1981, obtainable from Schweizerische Ferment AG, Basle, Switzerland), and also Celluclast 200 L is added in an amount of 20 g/kg dry matter (a commercial cellulase described in the leaflet NOVO enzymes, information sheet B 153 e-GB 1000 July, 1981, obtainable from NOVO INDUSTRI A/S, Novo Alle, 2880 Bagsvaerd, Denmark).
4. The contents of the tank is kept at 50°C during 24 hours with stirring.
5. The enzymes are inactivated by raising the pH value to 9.0 with 4N NaOH. The reaction mixture is kept for 30 minutes, and the pH value is then re-adjusted to 4.5 with 6N HCl.
6. The reaction mixture is centrifuged, and both the centrifugate and the sludge are collected.
7. The centrifugate from step 6 is check filtered on a filter press (the filter is washed with water before check filtration).
8. The check filtrate is ultrafiltered, diafiltered and once more ultrafiltered on a membrane with a cut-off value of 30,000 (DDS GR 60—P from De Danske Sukkerfabrikker), whereby the following parameters are used:

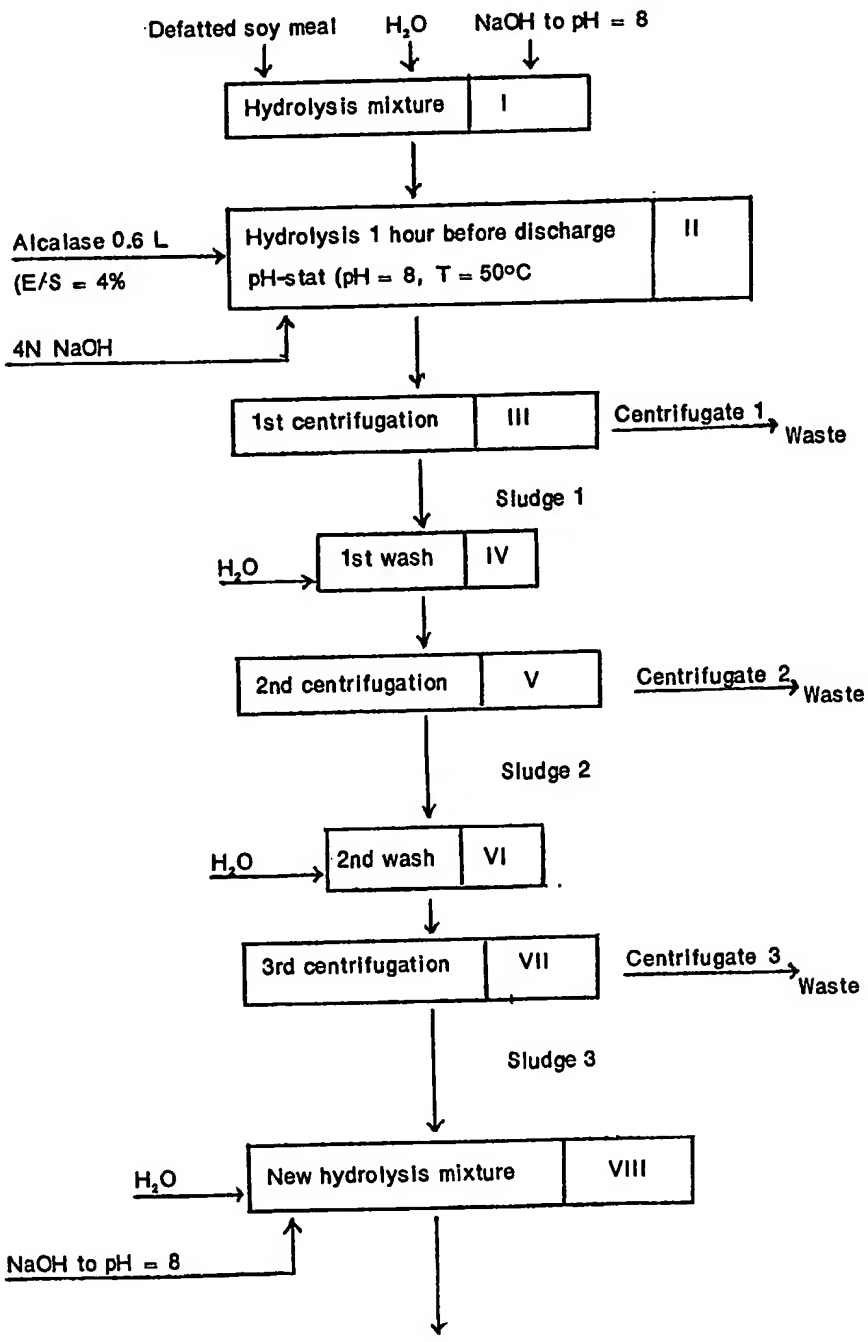
1. Ultrafiltration corresponding to a volume concentration of 6.
2. Diafiltration until the percentage of dry matter in the permeate is 0 (~ 0° Brix).
3. Ultrafiltration to around 15% dry matter in the concentrate.

The temperature is 50°C, pH is 4.5 and the average pressure is 3 bar.

9. The ultrafiltered concentrate is cooled to 5°C, and an equal volume of 96% ethanol is added.

10. The precipitate is collected by means of a centrifuge.
11. The precipitate is washed twice with 50% v/v ethanol in H_2O, corresponding to the volume of centrifugate from step 10, i.e. two centrifugations are performed.
12. The washed precipitate is redissolved in water with a volume which equals the volume of the ultrafiltered concentrate from step 9.
13. The liquid from step 12 is check filtered on a glass filter.
14. The clear filtrate containing pure SPS is lyophilized.

FLOW SHEET NO. 1



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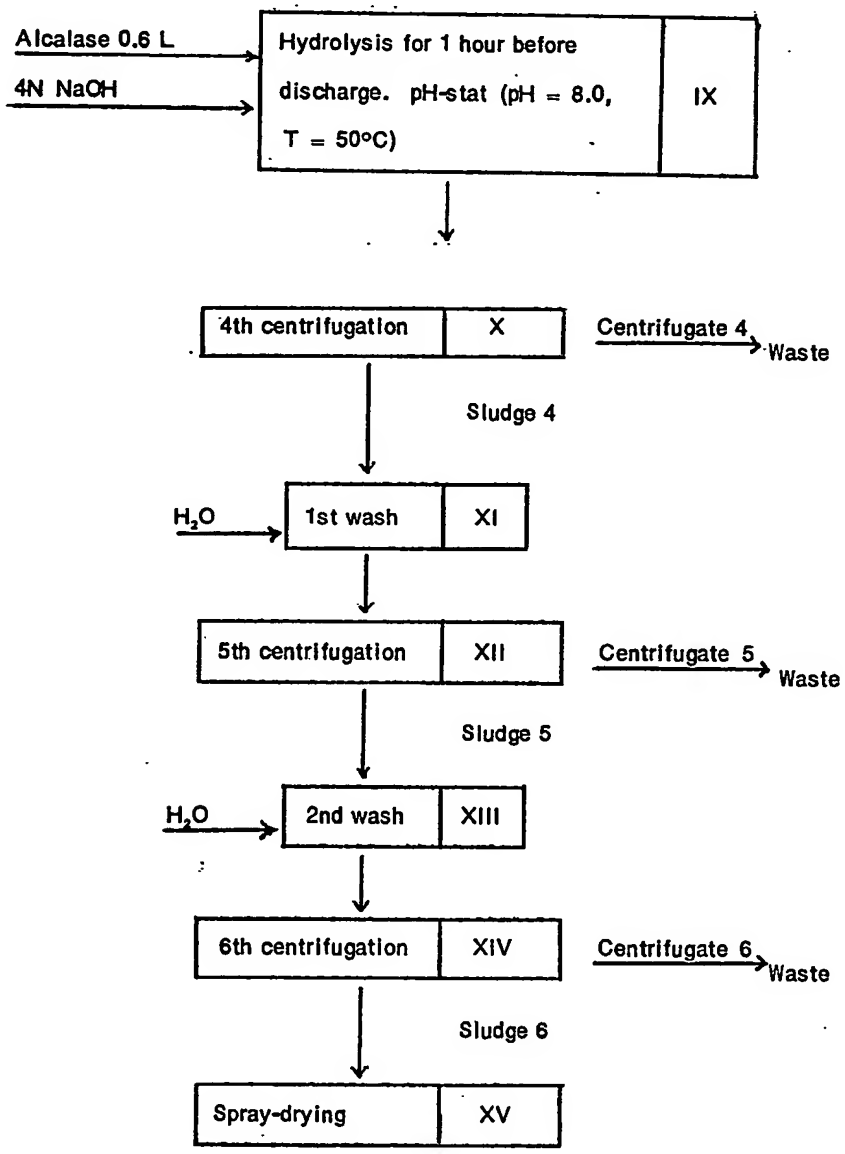

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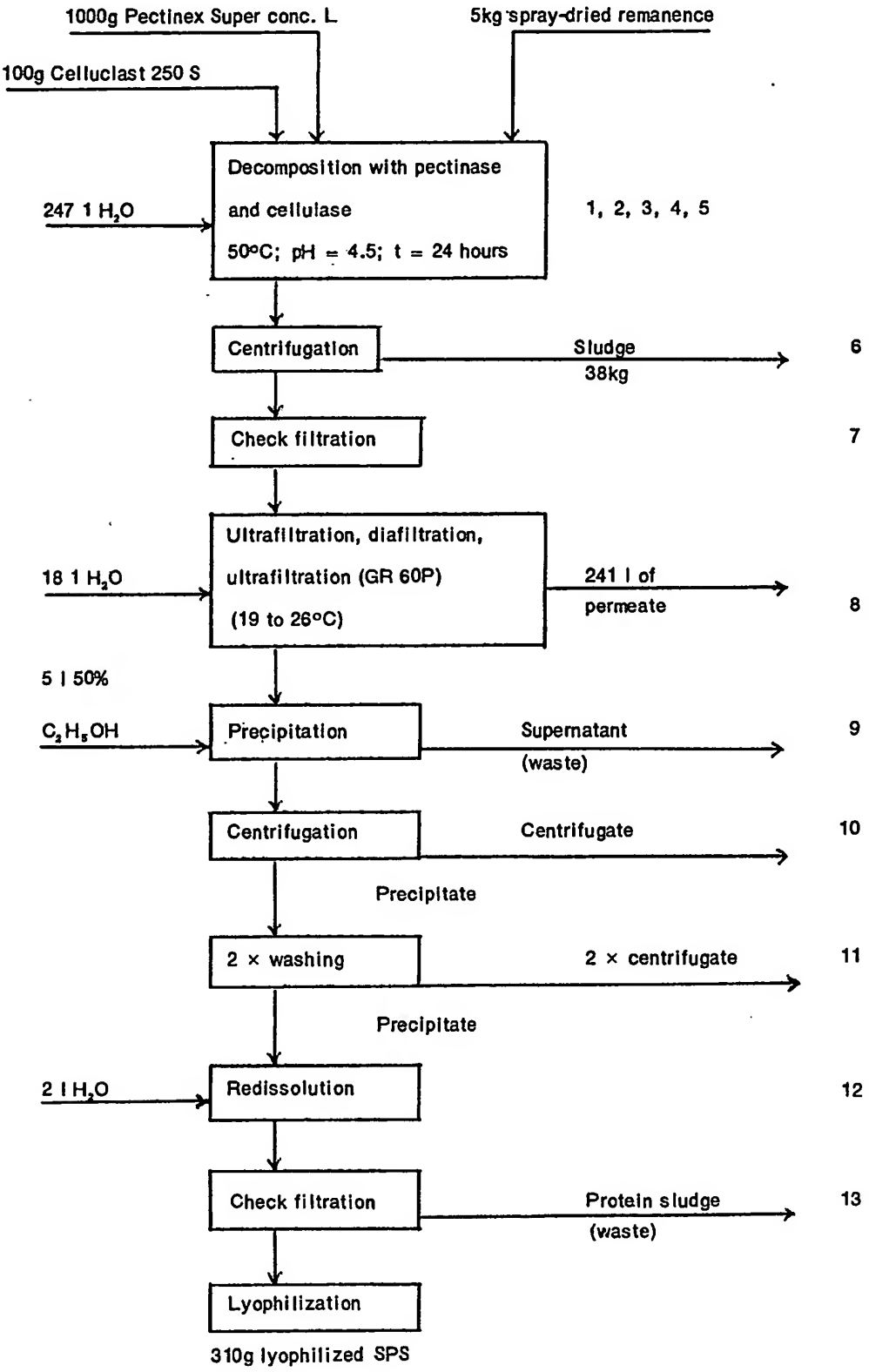


SECTION 2

CHARACTERIZATION OF SPS, ESPECIALLY MOLECULAR WEIGHT DISTRIBUTION THEREOF

By means of gel chromatography on HPLC equipment (Waters pump model 6000, Waters data module 730, and Waters refractometer R 401) the molecular weight distribution of the SPS, the production of which is carried out as indicated in this specification, is determined (Fig. 4). By means of the same method also the molecular weight distribution of the decomposition products of SPS by means of SPS-ase has been determined (Fig. 5). Furthermore, by means of the same method the binding effect between soy protein and SPS (Fig. 6) and the absence of binding effect between soy protein and SPS decomposed by means of the agent according to the invention (Fig. 7) has been demonstrated.

The calibration curve (the logarithm of the molecular weight plotted against R_f, where the R_f-value for glucose is arbitrarily defined as 1 and the R_f-value for a specific dextran is defined as the retention time for the dextran divided by the retention time for glucose) has been established by means of several standard dextrans with known molecular weights (T 4, T 10, T 40, T 70, T 110, T 500) from Pharmacia Fine Chemicals AB, Box 175, S—75104, Uppsala, Sweden. The R_f-value for the maximum of each dextran peak has been found, and the corresponding molecular weight has been calculated as

$$\sqrt{M_w \cdot M_n},$$

whereby $\overline{M}_w$ is the average value of the molecular weight according to weight and $\overline{M}_n$ is the average value of the molecular weight according to number. As an eluent for this chromatographic procedure 0.1 M $NaNO_3$ has been used. The columns used in the chromatographic procedure are 60 cm PW 5000 followed by 60 cm PW 3000 from Toyo Soda Manufacturing Co., Japan. In this manner the relationship between molecular weight and R_f for the above indicated dextrans has been established, vide Figure 3.

On the basis of Fig. 4 it can be calculated that SPS has a molecular weight distribution which gives rise to a value of $\overline{M}_w$ of around 5.4×10^5 and a value of $\overline{M}_n$ of around 4.2×10^4. Also, it appears from this figure that the chromatogram exhibits two distinct peaks at retention time 34.5 minutes (6%) corresponding to a molecular weight of around 5×10^6 and retention time 47.12 minutes (67%) corresponding to a molecular weight of around 4.9×10^4. Also, it appears from this curve that a shoulder exists between these two peaks at retention time 41.25 minutes (27%) corresponding to a molecular weight of 2.8×10^5.

After decomposition of SPS with SPS-ase the hydrolysis mixture was membrane filtered, and the filtrate was chromatographed. It was found that around 55% of SPS is decomposed to mono-, di- and trisaccharides, and that the remaining 45% are decomposed to a polymer with three peaks with the following molecular weights: 5×10^4, 10^4 and 4.4×10^3, vide Figure 5.

In order to demonstrate the binding effect between soy protein and SPS and the substantial reduction of binding effect between soy protein and SPS decomposed by means of an SPS-ase the following experiments have been performed.

3% SPS in 0.10 M acetate buffer at pH 4.5 is added to a slurry of soy isolate (Purina E 500) in order to generate a suspension with a ratio isolate/SPS of 10:1. This suspension is incubated for 18 hours on a shaking bath at 50°C. After incubation the suspension is centrifuged, and the clear supernatant is analyzed on HPLC as previously described. From Fig. 6 in comparison with Fig. 4 it appears that the SPS is completely adsorbed to the soy isolate.

The same procedure as indicated in the previous paragraph is performed with a 3% SPS solution hydrolyzed with an SPS-ase produced by means of CBS 101.43 (Fig. 7). A comparison between Fig. 7 and Fig. 5 shows that no compound in the hydrolyzed SPS with molecular weight below around 10^4 is adsorbed to soy isolate. The hydrolysis reduces the quantitative binding to around 10 to 15% in relation to the binding of SPS to soy protein.

An NMR-analysis of the SPS, the production of which is carried out as indicated in this specification reveals the following approximate composition of the SPS:

1) α-galacturonic acid in an amount of approximately 45%, whereby approximately 40% of the total amount of α-galacturonic acid is present as the methyl ester.
2) rhamnopyranose in an amount of approximately 20%,
3) galactopyranose in an amount of approximately 15%, and
4) β-xylopyranose in an amount of approximately 20%.

The constituents seem to be present in a structure comprising a rhamnoglacturonic backbone and side chains of xylose and galactose.

Complete acid hydrolysis of SPS (8 hours in 1 N H_2SO_4) and subsequent TLC analysis revealed that also minor amounts of the monosaccharides fucose and arabinose were present in the hydrolyzed SPS.

An HPLC analysis of the SPS decomposed by the SPS-ase enzyme complex formed by CBS 101.43 shows a powerful reduction of molecular weight. In accordance therewith the NMR-spectrum of the SPS decomposed as indicated above shows that the main part of the ester groups have disappeared and that also the content of xylose and galactose in the higher molecular weight material has

decreased. The NMR-spectrum of the part of the SPS decomposition product which precipitates by addition of one volume of ethanol to one volume of SPS decomposition product is similar to the NMR-spectrum of the SPS, with the above indicated modifications, concerning the ester groups and the content of xylose and galactose.

SECTION 3

DOCUMENTATION FOR THE FACT THAT SPS AND APS ARE DIFFERENT COMPOUNDS

APS was prepared as indicated in Agr.Biol.Chem., Vol. 36, No. 4, p. 544 to 550 (1972).

Now, this polysaccharide and SPS were hydrolyzed with different enzymes, whereafter the decomposition mixture was gel chromatographed on HPLC equipment, as indicated in section 2, "Characterization of SPS, especially molecular weight distribution thereof".

In more detail, the hydrolyses were carried out by treatment of 25 ml solution of either 2% APS or 2% SPS in 0.1 M acetate buffer of pH 4.5 with 10 mg KRF 68 or 30 mg Pectolyase. KRF 68 is an SPS-ase preparation, the preparation of which is described in example 1. The results appear from the following table.

Polysaccharide	Enzyme	HPLC gel chromatogram	Polysaccharide	
			Decomposed	Not decomposed
APS	Pectolyase	Fig. 8	X	
APS	KRF 8	Fig. 9	X	
SPS	Pectolyase	Fig. 10		X
SPS	KRF 68	Fig. 5	X	

SECTION 4

SCREENING FOR SPS-ASE PRODUCING MICROORGANISMS

The microorganism to be tested is incubated on an agar slant substrate with a composition which enables growth of the microorganism. After initial growth on the agar slant substrate the microorganism is transferred to a liquid main substrate, in which the main carbon source is SPS (prepared as indicated), in which the nitrogen source is NO_3^-, NH_4^+, urea, free amino acids, proteins or another nitrogen containing compounds, and which furthermore contains a mixture of necessary salts and vitamins, preferably in the form of yeast extract. The composition of the main substrate depends upon the microorganism genus, the principal issue being that the main substrate should be able to support growth and metabolism of the microorganism. When the growth has taken place in a suitable period of time, of the order of magnitude of 1 to 7 days, depending upon the growth rate of the microorganism in question, a sample of the fermentation broth is analyzed for SPS-ase according to the enzymatic SPS-ase determination described in this specification, or according to any other SPS-ase determination tailored to other specific uses of the SPS-ase than the use as a component of an agent for decomposition of soy remanence.

In order to achieve a more sensitive method for the determination of enzymatic activity the temperature could be lowered to 40°C and the incubation time could be raised to 20 hours during the determination of SPS-ase activity, whereby antibiotics should be added to the substrate in order to avoid infection.

By following this test method other SPS-ase producing microorganisms may be found, both belonging to the genus of Aspergillus and to other genera.

SECTION 5

CHARACTERIZATION OF SOME SPA-ASE FORMING MICROORGANISMS

According to the here indicated screening for SPS-ase producing microorganisms it has been found that the microorganisms listed in the upper part of the following table are SPS-ase producers. Also the table contains a strain belonging to the species Asp. japonicus which is not an SPS-ase producer.

SPS-ase producer		Species		Our identifying designation	Official identifying designation	First X deposition year
Yes	No	Asp. japonicus	Asp. aculeatus			
X			X	A 805	CBS 101.43;	1943 X
					DSM 2344	
X		X		A 1443	IFO 4408;	1950 X
					DSM 2346	
	X	X		A 1384	ATCC 20236;	1969 X
					DSM 2345	

A short identification of the above indicated strains can be found in the following culture catalogues:

List of Cultures 1978 Centraalbureau voor Schimmel-cultures, Baarn, The Netherlands.

Institute for Fermentation Osaka, List of Cultures, 1972, 5th Edition, 17—85, Fuso-honmachi 2-chome, Yodogawa-ku, Osako 532, Japan.

The American Type Culture Collection Catalogue of Strains I, 14th Edition 1980, 12301 Parklawn Drive, Rockville, Maryland 20852.

All the strains in the above indicated table correspond closely to the taxonomic description of the species Asp. japonicus and Asp. aculeatus appearing in The genus Aspergillus of Raper and Fennell, 1965 (vide especially pages 327 to 330).

SECTION 6

GENERAL DESCRIPTION OF OVERLAY TECHNIQUE ASSOCIATED WITH IMMUNOELECTROPHORESIS

A method designated the top-agar overlay technique has been developed by the applicant for identification of individual components of an enzyme complex by crossed immunoelectrophoresis with a polyspecific antibody against all enzyme components in the enzyme complex. The method is based on the fact that enzymes are still active after the specific enzyme-antibody binding, or otherwise stated that the active enzyme site is not identical to the site of the enzyme-antibody binding. The enzyme-antibody complexes precipitate as distinct arcs in the gel during the electrophoresis. The gel plate is covered with soluble SPS in a top-agar. After heating to 45°C for 20 hours in an atmosphere with a relative humidity of 100% the arc which possesses SPS-ase activity will appear as a clearing zone in the SPS cover after precipitation with a mixture of equal volume parts of ethanol and acetone when looked upon against a black background. Arcs which have no SPS-ase activity are left invisible.

SECTION 7

IMMUNOELECTROPHORETIC CHARACTERIZATION OF SPA-ASE WITH POLYSPECIFIC ANTIBODY AND OVERLAY

Rabbits were immunized with the SPS-ase containing enzyme complex obtained by fermentation of Aspergillus aculeatus CBS 101.43, as indicated in example 1 (KRF 68) and the polyspecific antibody was recovered in a manner known per se. By means of this polyspecific antibody a crossed immunoelectrophoresis of the enzyme complex obtained by fermentation of Asp. aculeatus CBS 101.43, as indicated in example 1 (KRF 68) was performed, as described in N.H. Axelsen et al., "A Manual of Quantitative Immunoelectrophoresis", 6' printing 1977. Reference is made to Fig. 11 which shows the arcs corresponding to the different proteins produced by the microorganism. By means of the previously described top-agar overlay technique it is found that the hatched area corresponds to SPS-ase.

If the previously indicated hypothesis comprising the assumption that SPS-ase consists of at least two enzymes is correct the hatched area is the area, in which all the enzymes responsible for the SPS-ase activity are present. If these enzymes in other embodiments of the invention should be separated by the immunoelectrophoresis in such a manner, that they do not cover any mutual area, a part of the SPS-ase activity can still be identified by means of immunoelectrophoresis with an overlay with both SPS and a commercial pectinase.

SECTION 8

PURIFICATION OF AN SPS-ASE PREPARATION

The purification of the SPS-ase preparation KRF 92 (vide example 1) was performed by ion exchange. The buffer is 50 mM Tris (tris-hydroxymethylaminomethane) which is adjusted to pH 7.0 with HCl. The column is K 5/30 from Pharmacia, Sweden. The ion exchange material is DEAE-trisacryl from LKB, Bromma, Sweden (300 ml). The flow rate is 100 ml/hour.

15 g of the SPS-ase preparation KRF 92 was dissolved in 450 ml of H_2O at 6°C, and all the following indicated operations were carried out between 6°C and 10°C. pH was adjusted to 7.0 with 1 M Tris. The column was equilibrated with the buffer, and then the SPS-ase sample was introduced onto the column. OD_{280} and the conductivity was measured on the eluate, reference being made to Fig. 12. Fraction 1 is the eluate which is not bound to the ion exchange material. Then the column is washed with 2000 ml buffer which gives rise to fraction 2. Now a 0 to 500 mM NaCl gradient is established, giving rise to fractions 3 to 9. All nine fractions were concentrated to 200 ml and dialyzed against water to a conductivity of 2 mSi by means of dialysis (Hollow Fiber DP 2 from Amicon, Massachusetts, U.S.A.). Then the nine fractions were freeze-dried. Only fractions 1 and 2 exhibited SPS-ase activity.

Fraction 1 was further purified by gel filtration 1.5 g of fraction 1 was dissolved in 10 ml 50 mM sodium acetate with pH 4.5 (500 mM KCl). The column is 2.5 × 100 cm from LKB. The gel filtration filling material is Sephacryl S—200 from Pharmacia, Sweden. The flow rate is 30 ml/hour. The fractions containing materials with molecular weights between 70,000 and 100,000, calibrated with globular proteins, contained an enzyme complex designated factor G which cannot decompose SPS when tested according to the qualitative agar test; however, SPS is decomposed according to the qualitative agar test when mixing factor G with a pectinase. It has been found that factor G is able to split off galactose, fucose, and some galacturonic acid from SPS, but the main decomposition product according to the HPLC analysis is still a high molecular product very much like SPS.

SECTION 9

pH-ACTIVITY DEPENDENCY, TEMPERATURE ACTIVITY DEPENDENCY AND STABILITY OF AN SPS-ASE

Fig. 13 shows the pH-activity dependency of the SPS-ase preparation KRF 68. From pH 2.7 to pH 3.5 a formic acid buffer system was used, and from pH 3.7 to 5.5 an acetate buffer system was used.

Fig. 14 shows the temperature activity dependency of the SPS-ase preparation KRF 68.

Fig. 15 shows the temperature stability of the SPS-ase preparation KRF 68.

SECTION 10

ENZYMATIC ACTIVITY DETERMINATIONS

The below indicated table is a survey of the different enzymatic activity determinations pertaining to the invention.

Enzyme	Kind of activity short designation	Definition of activity unit and description of enzymatic activity determination: Publicly available	Described later in this specification	Reference
SPS-ase	SPS-ase		X	
Remanence solubilizing	SRU	X		1
	SRUM-120		X	
Protease	HUT		X	
Cellulase	C_x	X		2
Pectinase	PU	X		3
	PGE	X		4
	UPTE	X		5
	PEE	X		6
Hemicellulase	VHCU	X		7

The references indicated in the last column of the above table are detailed in the below indicated table.

Reference No.	Identification of reference	Reference can be obtained from: NOVO INDUSTRI A/S, Novo Alle, 2880 Bagsvaerd, Denmark	Schweizerische Ferment AG, Basle, Switzerland	A library
1	Analyseforskrift AF 154/4 of 1981—12—01	X		
2	Analytical Biochemistry 84, 522—532 (1978)			X
	Analytical method AF 149/6—GB of 1981—05—25	X		
3	Determination of Pectinase Activity with Citrus Pectin (PU) of 23.3.1976		X	
4	Viskosimetrische Polygalacturonase-Bestimmung (PGE) of 10.11.77		X	

Reference No.	Identification of reference	Reference can be obtained from		
		NOVO INDUSTRI A/S, Novo Alle, 2880 Bagsvaerd, Denmark	Schweizerische Ferment AG, Basle, Switzerland	A library
5	Bestimmung der Pectintranseliminase (UPTE/g) of 24.Sept.1975		X	
6	Determination of the Pectinesterase activity (undated) with initials WJA/GW		X	
7	Analytical method AF 156/1—GB	X		

In relation to the cellulase activity determination it can be noted that the analysis was carried out as indicated in AF 149/6—GB and that the principles of the determination is explained in Analytical Biochemistry.

SECTION 10a

ENZYMATIC DETERMINATION OF SPA-ASE

The enzymatic determination of SPS-ase is carried out in two steps, i.e. a qualitative agar plate test, and a quantitative SPS-ase activity determination based on measurement of the amount of total liberated sugars. If the qualitative agar plate test is negative, the SPS-ase activity is zero, regardless of the value originating from the quantitative SPS-ase activity determination. If the qualitative agar plate test is positive, the SPS-ase activity is equal to the value originating from the quantitative SPS-ase activity determination.

I. Qualitative agar plate test.

An SPS-agar plate was prepared in the following manner. A buffer (B) is prepared by adjusting 0.3 M acetic acid to a pH-value of 4.5 by means of 1 N NaOH. 1 g of SPS is dissolved in 20 ml of B. 1 g of agarose (HSB LItex) is mixed with 80 ml of B and heated to the boiling point with stirring. When the agarose is dissolved the SPS-solution is slowly added. The resulting 1% SPS-agarose solution is placed in a water bath of 60°C. The plates are now cast by pouring 15 ml of the 1% SPS-agarose solution on a horizontal glass plate with dimensions 10 cm x 10 cm. Then 9 wells with a distance of 2.5 cm are punched out in the solidified layer of SPS-agarose. In each well a 10 μl of a 1% solution of the enzyme protein to be tested for SPS-ase activity is introduced. The plate is incubated for 18 hours at 50°C and with a relative humidity of 100%. Now still undecomposed SPS is precipitated by a solution of equal volume parts of ethanol and acetone. The SPS-ase agar plate test is positive for a sample placed in a specific well, if a clear annular zone appears around this well.

II. Quantitative SPS-ase activity determination test.

The purpose of this test is the determination of enzymatic activities, which are capable of decomposing SPS to such an extent that the decomposition products exhibit a strongly reduced or no adsorption or binding affinity to soy protein. Experiments have shown that that part of the SPS decomposition products which are not precipitated by a mixture of equal volumes of water and ethanol, do not have any adsorption or binding affinity to soy protein.

The SPS-ase determination is based on a hydrolysis of SPS under standard conditions followed by a precipitation of that part of SPS, which is not hydrolyzed with ethanol. After precipitation the content of carbohydrate, which is not precipitated, is determined by quantitative analysis for total sugar (according to AF 169/1, available from NOVO INDUSTRI A/S, 2880 Bagsvaerd).

The standard conditions are:
Temperature 50°C
pH: 4.5

Reaction time: control 210 minutes with substrate only, followed by 2 minutes with added enzyme: main value 212 minutes.

The equipment comprises:
Shaking water bath thermostated at 50°C
Whirlimixer stirrer
Centrifuge
Ice water bath

The reagents comprise:
Buffer: 0.6 M acetic acid in demineralized water (a) 1.0 M NaOH (b)
Substrate: The pH value of 50 ml of a is adjusted to 4.5 with b, then 4.0 g SPS are added, and after dissolution of the SPS the pH is readjusted to 4.5, and the volume is adjusted to 100 ml with deionized water.

Stop reagent: Absolute ethanol

1 SPS-ase activity unit (SAE or SPSU) is defined as the SPS-ase activity which under the above indicated standard conditions releases an amount of carbohydrate soluble in 50% ethanol equivalent to 1 μmol galactose per minute.

Even if the initial part of the enzyme standard curve is a straight line, it has to be noted that it does not intersect the (0.0) point.

SECTION 10b

ENZYMATIC DETERMINATION OF REMANENCE SOLUBILIZING ACTIVITY EXPRESSED AS SRUM 120

Principle

In the method for determination of hydrolysis activity the insoluble part of defatted, deproteinized, and dehulled soy flour is hydrolyzed under standard conditions. The enzyme reaction is stopped with stop reagent and the insoluble part is filtered off. The amount of dissolved polysaccharides is determined spectrophotometrically after acid hydrolysis according to AF 169/1, available from Novo Industri A/S, 2880 Bagsvaerd.

Carbohydrases with endo- as well as exo-activity are determined according to the method.

The substrate pertaining to this enzymatic determination is identical to the remanence substrate described for the SRU method. The substrate is dissolved as a 3% solution in the below indicated citrate buffer:

0.1 N citrate-phosphate buffer pH 4.5
5.24 g citric acid 1-hydrate (Merck Art 244)
8.12 g disodium hydrogen phosphate 2-hydrate (Merck Art 6580)
Ad 1 l demineralized H_2O
pH 4.5 ± 0.05
Stable for 14 days

The stop reagent has the following composition:
100 ml 0.5 N NaOH
200 ml 96% ethanol
To be kept in a refrigerator until use.

Standard conditions

Temperature	50°C
pH	4.5
Reaction time, sample	120 minutes
blank	5 minutes

Unit Definition

One soy remanence solubilizing unit (SRUM) 120 (M for manual) is the amount of enzyme which, under the given reaction conditions per minute, liberates solubilized polysaccharides equivalent to one micromole of galactose.

SECTION 10c

ENZYMATIC DETERMINATION OF PROTEOLYTIC ACTIVITY HUT MEASUREMENT

Method for the determination of proteinase in an acid medium.

The method is based on the digestion of denatured hemoglobin by the enzyme at 40°C, pH 3.2, for 30 minutes. The undigested hemoglobin is precipitated with 14% trichloroacetic acid (wt/v%).

All enzyme samples are prepared by dissolving them in 0.1 M acetate buffer, pH 3.2.

The hemoglobin substrate is prepared using 5.0 g of lyophilized, bovine hemoglobin powder, preserved with 1% Thiomersalate and 100 ml demineralized water which is stirred for 10 minutes, after

which the pH is adjusted to pH 1.7 with 0.33 N HCl.

After another 10 minutes of stirring, the pH is adjusted to pH 3.2 with 1N NaOH. The volume of this solution is increased to 200 ml with 0.2 M acetate buffer. This hemoglobin substrate must be refrigerated where it will keep for 5 days.

The hemoglobin substrate is brought to room temperature. At time zero, 5 ml of substrate is added to a test tube containing 1 ml of enzyme. After breaking for 1 second, the tube is placed in a 40°C water bath for 30 minutes. After exactly 30 minutes, 5 ml, 14% trichloroacetic acid is added to the reaction tube, which is then shaken and brought to room temperature for 40 minutes.

For the blank, the hemoglobin substrate is brought to room temperature. At time zero, 5 ml of the substrate is added to a test tube containing 1 ml of enzyme. After shaking for 1 second, the tube is placed in a 40°C water bath for 5 minutes. After exactly 5 minutes, 5 ml of 14% trichloroacetic acid is added to the reaction tube, which is then shaken and brought to room temperature for 40 minutes.

After 40 minutes, the blanks and samples are shaken, filtered once or twice through Berzelius filter No. 0, and placed in a spectrophotometer. The sample is read against the blank at 275 nm while adjusting the spectrophotometer against water.

Since the absorbance of tyrosine at 275 nm is a known factor, it is not necessary to make a tyrosine standard curve unless it is needed to check the Beckman spectrophotometer.

Calculations

1 HUT is the amount of enzyme which in 1 minute forms a hydrolysate equivalent in absorbancy at 275 nm to a solution of 1.10 microgram/ml tyrosine in 0.006 N HCl. This absorbancy value is 0.0084. The reaction should take place at 40°C, pH 3.2, and in 30 minutes.

$$HUT = \frac{\text{Sample-Blank}}{0.0084} \times \frac{\text{Vol. in ml}}{\text{reaction time in min.}}$$

$$HUT = \frac{\text{Sample-Blank}}{0.0084} \times \frac{11}{30} = (S—B) \times 43.65$$

$$HUT/g\ \text{enzyme} = \frac{(S—B) \times 43.65}{\text{g.enzyme in 1 ml}}$$

An investigation of the pH-stability dependency of the protease in KRF 68 performed by means of the HUT analysis with pH values from 2.0 to 8.0 showed that the stability of the protease above pH 8.0 was very small, vide Fig. 16.

In order to illustrate the invention reference is made to the following examples 1 to 8, where example 1 illustrates the production of SPS-ase, and where examples 2 to 8 illustrate the application of SPS-ase, with a soy based raw material in order to produce a purified vegetable protein. Other applications of SPS-ase are indicated in the section between example 8 and the survey of the figures.

Several fermentations with the here indicated strains of Asp. aculeatus and Asp. japonicus were performed in laboratory scale. Hereby preparations were obtained which contained SPS-ase according to the here indicated SPS-ase test. However, as rather large amounts of SPS-ase are required in order to run application tests, similar fermentations were run on a pilot plant scale, vide the following Example 1.

EXAMPLE 1

Production of an SPS-ase in pilot plant scale.

An SPS-ase was prepared by submerged fermentation of Aspergillus aculeatus CBS 101.43.

An agar substrate with the following composition was prepared in a Fernbach flask:

Pepton Difco	6 g
Aminolin Ortana	4 g
Glucose	1 g
Yeast extract Difco	3 g
Meat extract Difco	1.5 g
KH_2PO_4 Merck	20 g

Malt extract Evers	20 g
Ion exchanged H_2O ad	1000 ml

pH was adjusted to between 5.30 and 5.35. Then 40 g of Agar Difco was added, and the mixture was autoclaved for 20 minutes at 120°C (the substrate is named E-agar).

The strain CBS 101.43 was cultivated on an E-agar slant (37°C). The spores from the slant were suspended in sterilized skim-milk, and the suspension was lyophilized in vials. The contents of one lyophilized vial was transferred to the Fernbach flask. The flask was then incubated for 13 days at 30°C.

A substrate with the following composition was prepared in a 500 litre seed fermenter:

$CaCO_3$	1.2 kg
Glucose	7.2 kg
Rofec (corn steep liquor dry matter)	3.6 kg
Soy bean oil	1.2 kg

Tap water was added to a total volume of around 240 litres. pH was adjusted to around 5.5 before addition of $CaCO_3$. The substrate was sterilized in the seed fermenter for 1 hour at 121°C. Final volume before inoculation was around 300 litres.

The Fernbach flask spore suspension was transferred to the seed fermenter. Seed fermentation conditions were:

Fermenter type: Conventional aerated and agitated fermenter with a height/diameter ratio of around 2.3.
Agitation: 300 rpm (two turbine impellers)
Aeration: 300 normal litre air per minute
Temperature: 30 to 31°C
Pressure: 0.5 ato
Time: Around 28 hours

Around 28 hours after inoculation 150 litres was transferred from the seed fermenter to the main fermenter.

A substrate with the following composition was prepared in a 2500 litre main fermenter:

Toasted soy meal	90 kg
KH_2PO_4	20 kg
Pluronic (registered Trade Mark)	150 ml

Tap water was added to a total volume of around 900 litres. The toasted soy meal was suspended in water. pH was adjusted to 8.0 with NaOH, and the temperature was raised to 50°C. Thereafter around 925 Anson units of ALCALASE (registered Trade Mark) 0.6 L was added to the suspension. The mixture was held for 4 hours at 50°C and pH = 8.0 (Na_2CO_3 addition) with no aeration, zero ato and 100 rpm agitation. Thereafter the remaining substrate components were added and pH was adjusted to around 6.0 with phosphoric acid. The substrate was sterilized in the main fermenter for $1\frac{1}{2}$ hours at 123°C. Final volume before inoculation was around 1080 litres.

Then 150 litres of seed culture was added.

Fermentation conditions were:
Fermenter type: Conventional aerated and agitated fermenter with a height/diameter ratio of around 2.7.
Agitation: 250 rpm (two turbine impellers)
Aeration: 1200 normal litre air per minute
Temperature: 30°C
Pressure: 0.5 ato
Time: Around 151 hours

From 24 fermentation hours to around 116 fermentation hours pectin solution was added aseptically to the main fermenter at a constant rate of around 8 litres per hour. The pectin solution with the following composition was prepared in a 500 litre dosing tank:

Pectin genu [x]	22 kg
Phosphoric acid, conc.	6 kg
Pluronic (registered Trade Mark)	50 ml

[x] Genu pectin (citrus type NF from The Copenhagen pectin factory Ltd.)

Tap water was added to a total volume of around 325 litres. The substrate was sterilized in the dosing tank for 1 hour at 121°C. Final volume before start of dosage was around 360 litres. When this portion ran out, another similar portion was made. Total volume of pectin solution for one fermentation was around 725 litres.

After around 151 fermentation hours the fermentation process was stopped. The around 1850 litres of culture broth were cooled to around 5°C and the enzymes were recovered according to the following method.

The culture broth was drum filtered on a vacuum drum filter (Dorr Oliver), which was precoated with Hy-flo-super-cel diatomaceous earth (filter aid). The filtrate was concentrated by evaporation to around 15% of the volume of the culture broth. The concentrate was filtered on a Seitz filter sheet (type supra 100) with 0.25% Hy-flo-super-cel as a filter aid (in the following table referred to as filtration I). The filtrate was precipitated with 561 g of $(NH_4)_2SO_4$/l at a pH of 5.5, and 4% Hy-flo-super-cel diatomaceous earth is added as a filter aid. The precipitate and the filter aid are separated by filtration on a frame filter. The filter cake is dissolved in water, and unsoluble parts are separated by filtration on a frame filter. The filtrate is check filtered on a Seitz filter sheet (type supra 100) with 0.25% Hy-flo-super-cel as a filter aid (in the following table referred to as filtration II). The filtrate is diafiltered on an ultrafiltration apparatus. After diafiltration the liquid is concentrated to a dry matter content of 12.7% (in the following table referred to as dry matter content in concentrate).

A facultative base treatment for partial removal of the protease activity can be carried out at this stage. In case the base treatment is used it is carried out at a pH of 9.2 for 1 hour, whereafter the pH value is adjusted to 5.0.

Now the liquid is check filtered and filtered for the purpose of germ reduction and the filtrate is freeze-dried on a freeze-drying equipment from Stokes.

Four fermentations were carried out in the manner indicated below, whereby the strain used for the fermentation, the use of the facultative base treatment and other parameters were varied, as indicated in the following table.

Microorganism	Base treatment used	Base treatment not used	Preparation code	Concentration (%) of filter aid in connection with filtration I	Concentration (%) of filter aid in connection with the precipitation	Concentration (%) of filter aid in connection with filtration II	Dry matter content in concentrate	Remarks
CBS 101.43		X	KRF 68	0.5	5	0.2	28	
ATCC 20236		X	KRF 74	2.0	4	0.4	7.5	
IFO 4408		X	KRF 83	1.0	5	0.25	12.4	x)
CBS 101.43	X		KRF 92	0.25	4	0.25	12.7	

x) After germ reducing filtration the filtrate is concentrated by evaporation in a ratio of 1:2.3. A minor part of the concentrated filtrate was spray-dried, and the remaining part was freeze-dried.

In order to reduce the protease activity further, some of the above indicated preparations were treated as indicated below, whereby only one of the three alternatives A, B, and C was used.

A. 100 g SPS-ase preparation are dissolved in 1 litre of deionized water with stirring at 10°C ± 2°C. pH is adjusted to 9.1 with 4 N NaOH. This base treatment is carried out for 1 hour. The pH value is then adjusted to 4.5 with glacial acetic acid, and it is dialyzed against ice cold, deionized water to a conductivity of 3 mSi. Then freezing and lyophilization are carried out.

B. 500 g SPS-ase preparation are dissolved in 4 litre of deionized water with stirring at 10°C ± 2°C. pH is adjusted to 9.1 with 4 N NaOH. This base treatment is carried out for 1 hour. The pH value is then adjusted to 5.0 with glacial acetic acid. The obtained material is lyophilized.

C. 50 g SPS-ase preparation are dissolved in 400 ml of deionized water with stirring at 10°C ± 2°C. pH is adjusted to 9.1 with 4 N NaOH. This base treatment is carried out for 1 hour. Then pH is reduced to 5.7 with glacial acetic acid. The obtained material is lyophilized.

SPS-ase preparation used as starting material for base treatment	Base treatment used			
	A	B	C	Preparation code
KRF 68	X			KRF 68 BII
KRF 68		X		KRF 68 BIII
KRF 92			X	KRF 92 BI

The above indicated preparations are characterized by their activities of the enzymes relevant to the invention in the following table.

Enzyme activity per g		KRF 68	KRF 68 BII	KRF 68 BIII	KRF 74	KRF 83	KRF 92	KRF 92 BI
SAE	Plate test	+	+	+	–	+	+	+
	Quantitative test	350	301	349	0	168	476	430
SRU		737	507	481	142	683	626	757
$SRUM_{120}$		2125	1560	1720	578	753	1640	1030
HUT pH 3.2		67000	105	339	1630	12800	5960	397
C_x		8000	8044	9396	1320	8040	5700	3092
PU		10300000	9000000	8800000	840000	7500000	8400000	7600000
PGE		119400	72000	77700	4100	64600	60000	68800
UPTE		78100	83700	76900	15130	327000	44000	62400
PEE		840	910	770	370	690	1000	790
VHCU		1600000	1100000	1000000	65000	2200000	1100000	742000

EXAMPLE 2 (application example)

This example describes the production of a p.v.p. from a dehulled and defatted soy flour, "Sojamel 13" (commercially available from Aarhus Oliefabrik A/S). The dry matter content of this flour was 94.0% and the content of (N x 6.25) on a dry matter basis was 58.7%. The soy flour was treated with the SPS-ase preparations KRF 68 BII (Example 1) in the following manner:

85.2 g of the soy flour were suspended and kept stirred at 50°C in 664.8 g of water, and pH was adjusted to 4.5 by means of 7.5 ml of 6 N HCl. 50 g of a solution containing 4.00 g of said SPS-ase preparation was added, and the reaction mixture was then agitated for 240 minutes at 50°C. The mixture was then centrifuged in a laboratory centrifuge (Beckman Model J—6B) for 15 minutes at 3000 x g. The supernatant was weighed and analysed for Kjeldahl N and dry matter. The solid phase was then washed with a volume of water equivalent to the mass of supernatant obtained by the first centrifugation. This operation was performed twice. The solid phase was then freeze-dried, weighed and analysed for Kjeldahl N and dry matter at Qvist's Laboratorium, Marselis Boulevard 169, 8000 Aarhus C, Denmark. This laboratory is state authorized for analyses of fodder and dairy products. The results obtained in the experiment appear from Table 2.1:

TABLE 2.1
Results obtained

Component	Mass g	N x 6.25 %	Dry matter %	Yield of protein %	Yield of dry matter %
Soy flour	85.2	55.2	94.0	100%	100%
SPS-ase preparation	4.00	75.6	—	6.4%	—
1. Centrifugate	666	1.50	5.04	21.2%	42.0%
p.v.p.	44.5	87.5	95.7	82.7%	53.2%

Thus, a p.v.p. was obtained with a protein purity, i.e. N x 6.25 on dry matter basis, of 91.4%, and with a total yield of protein of 83%.

EXAMPLE 3 (application example)

This example was performed in order to compare the protein yields, the nutritional quality and some functional properties of soy protein products made by the following three procedures:

A: The traditional isoelectric precipitation for production of soy protein isolate.
B: The traditional isoelectric wash for production of soy protein concentrate.
C: The isoelectric wash according to the invention including a remanence solubilizing enzyme for production of p.v.p.

In order to generate a true comparison of the process according to the invention (C) with the conventional soy protein processes (A and B) the same raw material has been used in all three cases. Also the experiments have been conducted in such a manner that corresponding temperatures and treatment times are the same in all three cases. Only the pH-values were different due to the fundamental differences between the three experiments.

A. *The traditional isoelectric precipitation for production of soy protein isolate*

425.8 g of soy meal (Sojamel 13 produced by Aarhus Oliefabrik A/S) were extracted in 3574.2 g of tap water at 50°C. pH was adjusted to 8.0 with 20.1 g of 4 N NaOH. After stirring for 1 hour the slurry was centrifuged at 3000 x g for 15 minutes using four one litre beakers in a laboratory centrifuge (Beckman Model J—6B). The centrifugate I and the precipitate I were weighed. The precipitate I was re-extracted with water to a total weight of 4000 g. The temperature was kept at 50°C, pH adjusted to 8 with 4 N NaOH and the slurry kept stirred for one hour. A centrifugation and weighing of centrifugate II and precipitate II were performed as above. Samples were drawn from centrifugate I and II and precipitate II for Kjeldahl and dry matter determinations. Hereafter the centrifugates I and II were mixed and held at 50°C. The protein was then isoelectrically precipitated at pH 4.5 by means of 45 g of 6 N HCl. After stirring for 1 hour at 50°C the protein was recovered by centrifugation at 3000 x g for 15 minutes. The centrifugate III was weighed and analysed for Kjeldahl—N and dry matter. The solid phase III was weighed and washed with water in an amount corresponding to the weight of centrifugate I. The washing was carried out by stirring for one hour at 50°C. The washed protein was recovered by

centrifugation at 3000 x g for 15 minutes. The centrifugate IV and the solid phase IV were weighed. Centrifugate IV was analysed for Kjeldahl—N and dry matter. The solid phase was suspended in 1550 g of water at 50°C and pH was adjusted to 6.5 with 17 g of 4 N NaOH. The mixture was kept stirred for one hour and re-adjusted to pH = 6.5 if necessary. Finally the product was freeze-dried, weighed and analysed for Kjeldahl—N and dry matter. The mass balance calculations are shown in Table 3.1.

TABLE 3.1

Mass balance calculations of the traditional isoelectric precipitation for production of soy protein isolate.

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
Extraction:					
Soy flour	425.8	55.2	94.0	100.0	100.0
Water	3574.2	0	0	0	0
4 N NaOH	20.1	0	16.0	0	0.8
1. Centrifugation: Σ	4020.1	5.9	10.0	100.9	100.4
Centrifugate I	3141.0	4.4	6.9	58.8	54.1
Precipitate I	805.0	—	—	—	—
Re-extraction:					
Precipitate I	805.0	—	—	—	—
Water	3195.0	0	0	0	0
2. Centrifugation:					
Centrifugate II	3104.0	0.5	0.9	6.6	7.0
Precipitate II	820.0	9.1	17.2	31.7	35.2
Mixing and acidifying: Centrifugates I + II	6245.0	—	—	—	—
6 N HCl	45.0	0	21.3	0	2.4
3. Centrifugation: Σ	6290.0				
Centrifugate III	5650.0	0.3	1.9	7.2	26.8
Precipitate III	308.0	—	—	—	—
Washing:					
Precipitate III	308.0	—	—	—	—
Water	3141.0	0	0	0	0
4. Centrifugation: Σ	3449.0				
Centrifugate IV	3113.0	0.04	0.15	0.5	1.2
Precipitate IV	291.0	—	—	—	—

TABLE 3.1 (continued)

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
Neutralization:					
Precipitate IV	291.0	—	—	—	—
Water	1550.0	0	0	0	0
4 N NaOH	17.0	0	16.0	0	0.7
Drying:					
Powder	128.0	93.8	96.3	51.1	30.8

B. *The isoelectric wash for production of soy protein concentrate*

425.6 g of soy meal (Sojamel 13 produced by Aarhus Oliefabrik A/S) was washed in 3574 g of water at 50°C. pH was adjusted to 4.5 with 44.8 g of 6 N HCl. The washing was carried out for four hours by agitating. The slurry was then centrifuged at 3000 x g for 15 minutes in a laboratory centrifuge (Beckman Model J—6B) using four one litre beakers. The centrifugate I was weighed and analysed for Kjeldahl N and dry matter. The solid phase I was weighed and re-washed with water to a total weight of 4000 g. pH was re-adjusted to 4.5 with 1.7 g of 6 N HCl and the slurry was kept stirred for 30 minutes at 50°C. A centrifugation and weighing of centrifugate II and solids II were performed as above. The solid phase II was resuspended in 1575 g of H_2O at 50°C and pH was adjusted to 6.5 with 34.5 g of 4 N NaOH. The mixture was kept stirred at 50°C for one hour and re-adjusted to pH = 6.5 if necessary. Finally the protein product was freeze-dried, weighed, and analysed for Kjeldahl N and dry matter. The mass balance is shown in Table 3.2.

TABLE 3.2

Mass balance calculations of the isoelectric wash for production of soy protein concentrate.

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
Washing:					
Soy flour	425.8	55.2	94.0	100.0	100.0
Water	3574.0	0	0	0	0
6 N HCl	44.8	0	21.3	0	2.4
1. Centrifugation: Σ	4044.6	—	—	—	—
Centrifugate I	3150.0	0.6	3.2	8.0	25.2
Solids I	846.0	—	—	—	—
Re-washing:					
Solids I	846.0	—	—	—	—
Water	3154.0	0	0	0	0
6 N HCl	1.7	0	21.3	0	0.1

TABLE 3.2 (continued)

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
2. Centrifugation: Σ	4001.7				
Centrifugate II	3130.0	0.1	0.4	1.3	3.2
Solids II	863.0	—	—	—	—
Neutralization:					
Solids II	863.0	—	—	—	—
Water	1575.0	0	0	0	0
4 N NaOH	34.5	0	16.0	0	1.4
Drying:					
Powder	281.0	72.5	98.4	86.7	69.1

C. *The isoelectric wash including a remanence solubilizing enzyme for production of p.v.p.*

425.8 g of soy meal (Sojamel 13 produced by Aarhus Oliefabrik A/S) was washed in 3524.2 g of water at 50°C. pH was adjusted to 4.5 by use of 43.7 g of 6 N HCl. 24 g of the SPS-ase preparation KRF 68 BIII (Example 1) were solubilized in 26 g of water and added to the washing mixture. The washing was then carried out for four hours by agitation. Subsequently the purification was performed as described for B, the amounts of 6 N HCl, 4 N NaOH and water for resuspension being the only parameters with deviating values. The mass balance is shown in Table 3.3.

TABLE 3.3

Mass balance calculations of the isoelectric wash including a remanence solubilizing enzyme for production of p.v.p.

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
Washing:					
Soy flour	425.8	55.2	94.0	100.0	100.0
Water	3540.2	0	0	0	0
6 N HCl	43.7	0	21.3	0	2.3
SPS-ase:					
KRF 68 BIII	24.0	75.3	96.0	7.7	5.8
1. Centrifugation: Σ	4043.7	—	—	—	—
Centrifugate I	3420.0	1.7	5.2	24.7	44.4
Solids I	620.0	—	—	—	—

TABLE 3.3 (continued)

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
Re-washing:					
Solids I	620.0	—	—	—	—
Water	3380.0	0	0	0	0
6 N HCl	1.3	0	21.3	0	0.1
2. Centrifugation: Σ	4001.3				
Centrifugate II	3400.0	0.2	0.6	2.9	5.1
Solids II	577.0	—	—	—	—
Neutralization:					
Solids II	577.0	—	—	—	—
Water	1700.0	0	0	0	0
4 N NaOH	25.3	0	16.0	0	1.0
Drying:					
Powder	211.0	87.3[1)]	96.7[1)]	78.2	51.1
		86.9[2)]	97.0[2)]		

1) Analysed at Bioteknisk Institut, Holbergsvej 10, DK-6000 Kolding, Denmark.
2) Analysed at Qvist's Laboratorium, Marselis Boulevard 169, DK-8000, Aarhus C, Denmark.

Nutritional Properties

The amino acid compositions of the three protein products were determined, vide Table 3.4. The total content of essential amino acids, the chemical score and the essential amino acid index (EAAI) is calculated using the FAO reference pattern from 1957.

The trypsin inhibitor content of the three products was determined by means of the method described in A.O.C.S. Tentative Method Ba 12 to 75 (A.O.C.S. is an abbreviation for American Oil Chemists' Society). The results are shown in Table 3.5, which also includes the yields and the protein/dry matter ratio of the three products.

TABLE 3.4

Amino acid composition and nutritional evaluation of the three protein products A, B and C.

Amino acid	A. Soy protein isolate		B. Soy protein concentrate		C. Soy protein isolate (p.v.p.)	
	g/16g N	aas [1]	g/16g N	aas [1]	g/16g N	aas [1]
Non-essential:						
Aspartic acid	12.4	—	11.3	—	11.9	—
Serine	4.62	—	4.69	—	4.81	—
Glutamic acid	21.3	—	18.2	—	17.7	—
Proline	6.07	—	5.19	—	4.76	—
Glycine	4.13	—	4.26	—	4.33	—
Alanine	3.54	—	4.27	—	4.55	—
Histidine	2.83	—	2.78	—	2.50	—
Arginine	8.09	—	7.57	—	7.04	—
Essential:						
Isoleucine	4.87	> 100	4.97	> 100	5.19	> 100
Leucine	7.80	> 100	7.98	> 100	8.09	> 100
Lysine	6.24	> 100	6.09	> 100	5.57	> 100
Phenylalanine	5.47	>100 } >100	5.35	>100 } >100	5.17	>100 } >100
Tyrosine	3.38	>100 }	3.88	>100 }	4.44	>100 }
Cystine	1.29	64.5 } 56.4	1.32	66.0 } 60.2	1.44	72.0 } 65.5
Methionine	1.08	49.1 }	1.21	55.0 }	1.31	59.5 }
Threonine	3.10	> 100	3.60	> 100	3.97	> 100
Tryptophan	1.06	75.7	1.37	97.9	1.32	94.3
Valine	4.90	> 100	5.23	> 100	5.57	> 100
% total content of essential amino acids	38.36		41.31		42.21	
Chemical score	56.4%		60.2%		65.5%	
EAAI	86.7%		90.2%		91.3%	

[1] aas = amino acid score based on the FAO reference pattern (1957)

TABLE 3.5

Process characteristics and trypsin inhibitor content of the three protein products, A, B, and C.

		A. Soy protein isolate	B. Soy protein concentrate	C. Soy protein isolate (p.v.p.)
Process characteristics	Protein of dry matter	97.4%	73.7%	90.0%
	Protein yield	51.1%	86.7%	78.2%
Trypsin inhibitors TUI/g product		34,000	21,000	19,000
TUI/g protein		36,250	28,970	21,810

Functional Properties

Nitrogen solubility index (NSI) was determined in a 1% protein dispersion at pH = 7.0 in 0.2 M NaCl and in distilled water respectively. After stirring for 45 minutes with a magnetic stirrer the suspension was centrifuged at 4000 x g for 30 minutes, and the supernatant was analysed for nitrogen. The nitrogen solubility was calculated as (soluble N%/total N%). The results of this evaluation on the three products are shown in Table 3.6.

Emulsifying capacity was determined three times on each product by a slightly modified Swift titration. 4.0 g of (N x 6.25) of the product was blended in 250 ml of 0.5 M NaCl with a Sorval Omnimixer at low speed. 50 ml of the suspension were transferred to a glass blender jar and 50 ml of soy bean oil were added. Hereafter the total mixture was weighed. The oil-water mixture was then homogenized at 10,000 rpm with the jar in an ice-bath. A supplementary amount of soy bean oil was added at a rate of 0.3 ml per second until the emulsion collapses. The total amount of oil added before the "end point" was found by weighing.

Emulsifying capacity was calculated as ml oil per gram protein (N x 6.25). The density of the oil was taken as 0.9 g/ml.

The average results of the determination of emulsifying capacity on the three products are shown in Table 3.6.

Whipping expansion was determined in a 3% protein solution at pH = 6.5. 250 ml of the aqueous dispersion of the protein samples were whipped at speed III for 4 minutes in a Hobart mixer (model N—50) mounted with a wire whip. The whipping expansion was calculated according to the formula

$$\text{Whipping expansion} = \frac{V—250}{250} \times 100\%,$$

where V = final whip volume in ml.

V was measured by refilling the mixer jar with water. Duplicates were performed for each of the three samples. The average results are shown in Table 3.6.

Foam stability was determined as the ratio between the amount of foam left after draining for 30 minutes and the original amount of foam. A gram of foam produced by the method above was introduced into a plastic cylinder (diameter 7 cm, height 9 cm) having a wire net with a mesh size of 1 mm x 1 mm. The cylinder was placed on a funnel on top of a glass cylinder and the weight (B) of drained liquid in the glass cylinder is determined. The foam stability FS is defined by the equation

$$FS = \frac{A—B}{A} \times 100\%$$

The results of the determination is shown in Table 3.6.

The gel strength is in this specification defined as the Brookfield viscosity measured by means of T-spindles on a Brookfield Helipath stand. The gels were made by heat treatment of 12% protein suspensions in 0.5 M NaCl. The heat treatment was performed in closed cans with a diameter of 7.3 cm and a height of 5.0 cm placed in a water bath maintained at 80°C and 100°C each for 30 minutes. The cans were cooled and thermostatted to 20°C before they were opened and measured. The results of the measurements are shown in Table 3.6.

TABLE 3.6

Functional properties of the three protein products A, B, and C.

Functionality	A. Soy protein isolate	B. Soy protein concentrate	C. Soy protein isolate (p.v.p.)
% NSI in 0.2 M NaCl	39.5	20.3	25.6
% NSI in water	53.9	25.1	28.6
Emulsifying capacity: ml oil/g(N×6.25)	218	182	354
Whipping expansion %	120	120	340
Foam stability %	50	50	20
Gel strength; [poise] 80°C (0.5 M NaCl)	1.7×10^3	1.2×10^4	3.3×10^2
100°C (0.5 M NaCl)	2.0×10^4	4.0×10^4	1.3×10^4

EXAMPLE 4 (application example)

A p.v.p. was produced according to the procedure described in Example 3 C. except that the cellulose activity was partially derived from Trichoderma reseei. The commercial cellulose preparation CELLUCLAST produced by Novo Industri A/S was treated with a base at low temperature in the following manner. The pH value of a 10% CELLUCLAST solution in water was adjusted to 9.2 with NaOH, and the thus resulting solution was cooled to 5°C. After 1 hour at this pH and this temperature the pH was re-adjusted to 4.7 with 20% acetic acid. This solution was kept at 5°C overnight and then sterile filtered. The filtrate was freeze-dried. 4 g of the freeze-dried product was added together with the SPS-ase preparation KRF 68 BIII (Example 1). The two enzymes were solubilized in 172 g of water before addition to the washing mixture. The mass balance determinations of this example is shown in Table 4.1.

The experiment demonstrates that this particular SPS-ase preparation already contains an efficient cellulase as addition of CELLUCLAST does not seem to effect the protein/dry matter ratio. However, other SPS-ase preparations may contain less cellulase, e.g. KRF 92, vide the table immediately preceding Example 2.

TABLE 4.1

Mass balance determinations of the isoelectric wash including an SPS-ase preparation and CELLUCLAST (registered Trade Mark) for production of p.v.p.

Operations and fractions	Mass of fraction g	Protein % (N x 6.25)	Dry matter %	Yield of protein %	Yield of dry matter %
Washing:					
Soy flour	425.8	55.2	94.0	100.0	100.0
Water	3546.2	0	0	0	0
6 N HCl	43.1	0	21.3	0	2.3
SPS-ase:					
KRF—68—B—III	24.0	75.3	96	7.7	5.8
CELLUCLAST	4.0	43.6	96	0.7	1.0
Centrifugation: Σ	4043.1	—	—	—	—
Centrifugate I	3382.0	1.9	5.5	27.3	46.5
Solids I	661.0	—	—	—	—
Re-washing:					
Solids I	661.0	—	—	—	—
Water	3339.0	0	0	0	0
6 N HCl	0	0	0	0	0
2nd centrifugation: Σ	4000.0				
Centrifugate II	3414.0	0.2	0.7	2.9	6.0
Solids II	582.0	—	—	—	—
Neutralization Solids II	582.0	—	—	—	—
Water	1691.0	0	0	0	0
4 N NaOH	25.3	0	16.0	0	1.0
Drying:					
Powder	206.0	88.8	98.9	77.8	50.9

EXAMPLE 5 (application example)

A p.v.p. was produced according to the method described in Example 3 C. except that all masses were scaled down with a factor of 5, and that the reaction mixture was cooled to about 5°C prior to the centrifugation. On the basis of the analytical results in relation to the centrifugates a theoretical yield of precipitated protein is obtained, as shown in Table 5.1.

TABLE 5.1

Theoretical protein yields obtained in the production of p.v.p.

Fractions	Mass g	Protein (N < 6.25) %	Yield of protein %	Example 3 C. Protein (N x 6.25) %	Example 3 C. Yield of protein %
Soy flour	85.2	55.2	100	55.2	100
SPS-ase KRF—68 B—III	4.8	75.3	7.7	75.3	7.7
1st centrifugate	639	0.99	13.5	1.7	24.7
2nd centrifugate	595	0.13	1.6	0.2	2.9
p.v.p.	—	87.2[a]	92.6[b]	87.1	80.1[b]

[a] Average of 87.5 (Bioteknisk Institut) and 86.9 (Qvist's Laboratorium); dry matter is 97.6 and 98.0%, respectively.
[b] Calculated as total mass of protein — protein lost is centrifugates.

EXAMPLE 6 (application example)
Demonstration of the protein binding of SPS

40 grams of (N x 6.25) from a commercial soy protein isolate (Purina 500 E from Ralston Purina) was dissolved in 680 g of water. The mixture was heated in a water bath to 50°C, and pH was adjusted to 4.50 with 6 N HCl. 90 g of this mixture was transferred to 5 x 250 ml Erlenmeyer flasks, and 10 g of aqueous solutions containing respectively 0 g, 0.2 g, 0.4 g, 0.8 g and 1.6 g of the SPS produced as described previously in this specification was added. The flasks were then held under stirring with a magnet in a water bath at 50°C for 240 minutes.

Hereafter the slurries were centrifuged at 3000 x g for 15 minutes, and the centrifugates I were analysed for Kjeldahl—N and dry matter. The solid phases were washed in water at room temperature and re-centrifuged. This procedure was repeated. Then the solids were dispersed in 50 ml of water, and pH was adjusted to 6.50 by drop-wise addition of 6 N NaOH. The neutralized products were freeze-dried and analysed for Kjeldahl—N and dry matter. Based on the analysis shown in Table 6.1, the protein recovery and the percentage of SPS which has been bound to the protein are calculated by means of the formulas shown in relation to Table 6.2.

This example demonstrates that the SPS is bound firmly to the protein so that the protein/dry matter ratio decreases with increasing content of SPS. An SPS content comparable to about 0.4 g in 10 g of water added to 5 g of protein isolate is the protein/SPS ratio present in the soy flour.

The % binding of SPS is a calculated value. The % binding of SPS decreases due to saturation of the protein with regard to SPS at the low protein/SPS ratios.

TABLE 6.1

Measurements according to Example 6

Ratio Protein/SPS	Centrifugates I		Dried precipitate			
	% N	% DM	% N	% N x 6.25	% DM	$\frac{\% \text{ N} \times 6.25}{\text{DM}}$
∞	0.068	0.62	13.2	82.5	93.1	88.6
25	0.045	0.49	13.4	83.8	97.3	86.1
12.5	0.038	0.45	13.0	81.3	97.9	83.0
6.25	0.031	0.45	12.6	78.8	98.1	80.3
3.125	0.026	0.61	11.8	73.8	97.9	75.3

TABLE 6.2

Protein recovery and % binding of SPS

Ratio Protein/SPS	% recovery of protein [1]	% binding of SPS [2]
∞	91.5	0
25	94.4	77
12.5	95.3	90
6.25	96.1	70
3.125	96.8	60

1) % recovery of protein = $\left[1 - \frac{\text{NC 1} \times 6.25}{5}\right] \times 100,$

where NC 1 = % N in centrifugate I

2) % binding of SPS = $\left[\frac{5 \times (\%\text{ recovery of protein})}{(\%\text{ P/H})} - \frac{5 \times (\%\text{ recovery of protein})}{(\%\text{ P/H})\infty}\right] \times 100,$

where [5/ratio of SPS]

(% P/H) is the protein/dry matter ratio in the dried precipitate, and
(% P/H)∞ is for the precipitate without addition of SPS.

EXAMPLE 7 (application example)

This example describes the production of a p.v.p. using the SPS-ase preparation KRF 92 B—I in a dosage of 5% of the dry matter. The manner of production was exactly as in Example 3 C, except that all masses were scaled down with a factor of 5. The p.v.p. was analysed as described in Example 2. The results obtained in the experiment appear from Table 7.1.

TABLE 7.1

Results obtained in Example 7

Component	Mass g	(N x 6.25) %	Dry matter %	Yield of protein %	Yield of dry matter %
Soy flour	85.2	55.2	94.0	100	100
Enzyme preparation	4.0	71.2	—	6.1	—
1st centrifugate	632	1.88	5.44	25.3	43.0
2nd centrifugate	673	0.30	0.80	4.3	6.7
p.v.p.	39.8	85.6[a]	98.1[a]	71.9	48.8
		84.4[b]	98.1[b]		

[a] Analysed at Bioteknisk Institut, Holbergsvej 10, DK—6000 Kolding
[b] Analysed at Qvist's Laboratorium, Marselis Boulevard 169, DK—8000 Aarhus C.

EXAMPLE 8 (application example)

This example demonstrates the effect of pretreating the soy meal by jet cooking before the production of p.v.p.

Pretreatment

A slurry of soy meal in water consisting of 10 kg soy meal (Sojamel 13 producted by Aarhus Oliefabrik A/S) per 100 kg was pumped through a steam-ejector (type Hydroheater B—300) and mixed with steam of 8 Bar in such amount and by such flow that a final temperature of 150°C could be maintained for 25 seconds in a tubular pressurized reactor. Hereafter the pressure was released in a flash chamber (a cyclone) and from here the slurry was sent through a plate heat exchanger and cooled to about 50°C. The cooled slurry could be used directly for production of p.v.p. according to the invention, but in this case the slurry was spray-dried at an inlet temperature of 200°C and at an outlet temperature of 90°C. The pretreated product was found to have a dry matter content of 96.5% and a protein content of 56.9% (N x 6.25).

Production of p.v.p.

This production was carried out in the following way:

70 g of dry matter of the jet cooked and dried soy flour was suspended and kept stirred at 50°C in 560 g of water, and pH was adjusted to 4.50 by means of 6.5 ml of 6 N HCl. 6 x 90 g of this suspension was transferred to six 250 ml Erlenmeyer flasks and kept stirred on a 50°C water bath by means of magnetic stirrers. To each flask were added 10 g of a solution containing respectively 0 g; 0.025 g; 0.050 g; 0.10 g; 0.20 g, and 0.40 g of the SPS-ase preparation KRF—68—B—III. The reaction mixtures were then agitated for 240 minutes at 50°C. Then a centrifugation at 3000 x g for 15 minutes was carried out.

The supernatant was then analysed for Kjeldahl—N and the solid phase was washed with water at equal volumes and centrifuged. This procedure was performed twice. The solid phase was then freeze-dried and analysed for Kjeldahl—N and dry matter.

A similar experiment was carried out with an untreated soy meal (Sojamel 13 from Aarhus Oliefabrik A/S) as a starting material. In this case the enzyme substrate ratios were 0; 1%; 2%; 3%; 4% and 8%.

Based on the protein content of the supernatants the percentage of recovered protein can be calculated. The yield of protein is based on the assumption that the enzyme product is 100% solubilized after the reaction. The table below shows the results obtained by both experiments.

TABLE 8.1

	Cooked soy meal		Untreated soy meal	
E/S %	Protein yield %	Protein of dry matter %	Protein yield %	Protein of dry matter %
0	92.9	76.5	90.7	73.9
0.25	90.1	86.6	—	—
0.50	89.3	88.7	—	—
1.0	88.1	89.7	87.1	86.2
2.0	86.6	91.7	85.7	88.1
3.0	—	—	84.3	89.5
4.0	84.7	92.2	82.6	90.9
8.0	—	—	76.2	91.1

Table 8.1 Protein yields and protein dry matter ratio for p.v.p. produced from cooked or raw soy meal.

In relation to either the extraction (isolation) processes for materials other than proteins or to the liquefaction processes and thereto related processes, reference is made to the general process scheme for applications as shown in Flow Sheet No. 3.

The substrate may be one or more carbohydrates present in a raw material, or it may be the entire raw material.

This substrate may be subjected to a pre-treatment of chemical or physical nature, as later exemplified, e.g. acid or alkaline treatment, soaking or steeping, and/or cooking with or without steam.

The raw material may be macerated, chopped, wet milled, and/or homogenized (all these treatments being designated homogenization in Flow Sheet No. 3), with or without water additions, and other additives may be added during this step. The homogenization may be carried out with different efficiencies, e.g. different pressures being a fraction only of the maximum pressure given for the specific homogenizer used. Different additives may be added before or during homogenization, as symbolized by b_1, $b_2 \ldots b_n$ in Flow Sheet No. 3.

The reaction process including the SPS-ase preparation is carried out under specified conditions, e.g. temperature, pressure, time, pH, and enzyme dosage; also recommendations regarding the reactor used (e.g. batch, plug-flow) and the stirring, if necessary, are relevant. A set of additives can be given for different raw materials, indicated as c_1, c_2, $\ldots c_n$ in Flow Sheet No. 3.

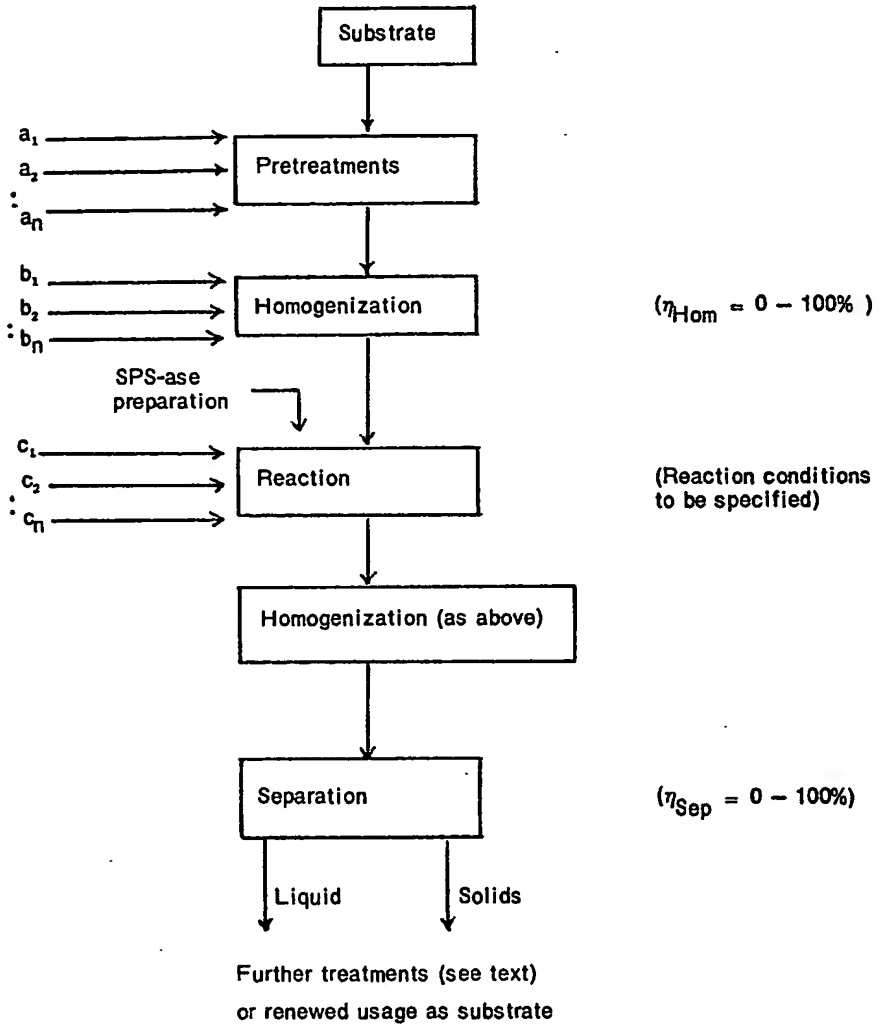
Also the separation processes may be carried out with different efficiencies. During many processes the separation is omitted or facilitated, e.g. when the raw material is totally liquefied. Different separation equipments may be used (e.g. centrifuges, filters, ultrafiltration equipment, hydrocyclones, thickeners, sieves or screens, or simple decanters).

The separation efficiency is defined as the proportion between the absolute sludge content in the solids phase and the absolute sludge content of the reaction mixture.

The liquid or the solid phases obtained may be further treated, e.g. concentrated, dried, or solvent extracted, to remove certain components like fat or oil, fermented for production of biomass, alcohol, or other products (enzymes, antibiotics, or other valuable components).

Also the obtained products may be returned to repeated treatment through the process scheme.

FLOW SHEET NO. 3



In the following some examples of applications of SPS-ase preparations are given, and a survey of these applications appears from the following list.

Also, in the adjoining Table I certain characteristics in relation to Flow Sheet No. 3 are listed.

List of exemplifying applications of SPS-ase preparations

Kind of SPS-ase preparation		Reference No.	
SPS-ase preparation essentially free of one or more unwanted enzyme activities		A 1	Extraction of starch from corn, wheat and potatoes
		A 2	Extraction of lipids from plant material
		A 3	Extraction of etheric oils from plant materials
		A 4	Extraction of natural coloring agents from plant material
		A 5	Extraction of rubber from the guayul bush
Unmodified SPS-ase preparations	Total liquefaction or similar treatment	Ba 1	Production of a milk substitute for domestic animals
		Ba 2	Production of saccharified starch containing raw materials
		Ba 3	Total liquefaction of pears and other fruits
		Ba 4	Production of juice by treatment of fruits and vegetables
		Ba 5	Treatment in relation to extraction or pressing of sugar cane or sugar beet
		Ba 6	Production of soy milk
		Ba 7	Treatment in order to increase the recoverable amount of coffee solubles.
Unmodified SPS-ase preparations	Processing aids	Bb 1	Prevention and/or decomposition of apple haze
		Bb 2	Use as a clarifying agent for white wine
		Bb 3	Production of ISSPH or other vegetable protein hydrolyzates
		Bb 4	Mashing enzyme in the brewing industry
		Bb 5	Enzymatic additive for use during their fermentation and/or storage
		Bb 6	Almon skin release agent
	Other applications	Bc 1	Decomposition of different waste materials
		Bc 2	Saccharification and simultaneous fermentation
		Bc 3	Decomposition of cellulose
		Bc 4	Application as a baking aid
		Bc 5	Improvement of alcohol yield and yield of biomass during fermentation of sulfite liquor from paper production
		Bc 6	Dewatering of biological sludge products
		Bc 7	Silage aid

TABLE I

Ref.	Substrate	Additives			1′ homo-genization η Hom%	2′ homo-genization η Hom%	Separation η Sep %	Liquid phase	Further treatments	
		a_i	b_i	c_i					Solid phase	Combined phase (no separation)
A 1	corn	water SO_2	water	NaOH or HCl	20—50	10—30	30—50	(Contains germ) washing, oil recovery	Washing operations	—
A 2	corn germ	—	—	NaOH or HCl	0	0	100	(Oil + water) purification	(waste)	—
Ba 1	Soy flour (defatted)	water	—	HCl	0	0	0	—	—	pasteurisation concentration, spray drying
Ba 2	sweet potatoes	water	Terma-myl	NaOH or HCl	—	—	0	—	—	yeast for alcohol fermentation, distillation
Ba 5	sugar beets	water	—	NaOH or HCl	10%	—	100	crystal-lization	(waste)	—
Bc 1	tofu or soy milk residue	water	—	HCl	10%	—	0	—	—	yeast for alcohol fermentation, distillation

A 1. Extraction of starch from corn, wheat, and potatoes.

Extraction of starch from corn, wheat, potatoes, and other starch containing plants is carried out by one or more of the steps: steeping, wet milling, and separation. Application of an SPS-ase preparation with essentially no amylolytic activity will provide the following advantages, corn being used as an example:

1. The starch liberation will be facilitated during a shorter steeping period,
2. The water consumption can be reduced,
3. The liberation of corn germ would be facilitated without liberation of corn germ oil,
4. The protein can be obtained in a higher purity,
5. The recovery of corn steep water will be facilitated.

A 2. Extraction of lipids from plant material.

As the lipids in plant materials are trapped inside the cells and usually bound to proteins, lipids may be extracted in aqueous phase by treatment with an SPS-ase preparation which is essentially free from lipases. Thus, corn germ oil normally is isolated by hexane extraction of the dried corn germs. However, the drying operation is superfluous if the wet corn germs are treated with an SPS-ase preparation of the above indicated kind. Likewise, the extraction of olive oil in aqueous phase can be improved, if the enzyme used for the enzymatic treatment is a SPS-ase preparation of the above indicated kind, vide e.g. Food, Pharmaceutical and bioengineering, No. 172, vol. 74, p. 93 to 94. Also, aqueous extraction of e.g. soy oil, rape seed oil and sunflower oil may be improved in a similar manner.

A 3. Extraction of etheric oils from plant materials.

If vegetable materials containing etheric oils are extracted with an aqueous solution of an SPS-ase preparation which is essentially free from enzymatic activity capable of decomposing or otherwise changing the etheric oils, the etheric oils will be recovered in high yields at a very low cost.

A 4. Extraction of natural colouring agents from plant material.

If vegetable materials containing colouring agents, e.g. beetroots containing the red colouring agent betanin or the colouring agent in cranberries, are treated with an SPS-ase preparation which is essentially free from enzyme activities capable of decomposing or otherwise changing the colouring agents, the colouring agents will be recovered in high yields at a very low cost.

A 5. Extraction of rubber from the guayul bush.

Another example of a substrate for an SPS-ase preparation which is essentially free from enzymatic activity capable of degrading native rubber is the cell wall material in roots and branches of the guayul bush.

Ba 1. Production of a milk substitute for domestic animals, preferably a calf milk substituted.

By a total liquefaction in aqueous medium of soy beans, sunflower seeds, cotton seeds, faba beans, or field peas a calf milk substitute which is soluble in cold water at a pH value of around 4.5 can be produced. Using the starch containing raw materials like faba beans or field peas a starch liquefaction by means of an alpha-amylase has to be accomplished before, after or simultaneously with a treatment with an SPS-ase which finally solubilizes the non starch polysaccharides present as the structural material in the cell walls. A detailed example is shown below using faba beans, and as regards soy beans reference is made to table I. The pre-treatment of said soy beans may preferably be a jet-cooking which improves the solubilization of the remanence.

EXAMPLE Ba 1.1

15 kg of faba bean flour (Farine de Feves from GRANDES MINOTERIES A FEVES DE FRANCE, Paris) were suspended in 35 litres of water. 75 g of Termamyl 60 L and 18 g of $CaCl_2$ were added. The suspension was heated to 95°C using a steam jacketed vessel while stirred. The suspension was then treated at this temperature for 60 minutes. Hereafter pH was adjusted to pH = 4.5, and the product was cooled to 50°C. 300 g of the SPS-ase preparation KRF 68 was solubilized in 1 litre of water and added. The reaction was carried out for 440 minutes. When 10 g of Fungamyl 800 L is included the starch fraction mainly will be converted to disaccharide (maltose). Hereafter the reaction mixture was pasteurized at 90°C for 2 minutes. An aliquot of the product was then freeze dried and used for stability tests. The sample was then solubilized at 10% dry matter, and the solution of the product could be kept stable without sedimentation for days.

Melted fat or oil may easily be emulsified in the product whereby a final composition very similar to cow milk may be obtained. An emulsion containing 3.5% oil (soy bean oil) could also be kept stable without sedimentation for days.

EXAMPLE Ba 1.2

Soy flour (Sojamel 13) was jet cooked at 150°C for 25 seconds as described in example 8. The jet cooked soy flour was spray-dried and used for further studies described in the following.

A: 50 g of the jet cooked soy flour was mixed with 450 g of water, and pH was adjusted to 4.5 with 4.1 ml 6 N HCl. The mixture was then heated to 45°C in a water bath, and 0.250 g of the SPS-ase preparation KRF—68 was added to the heated mixture which was then reacted for 5 hours with stirring. Thereafter the mixture was heat treated at 80°C for 2 minutes in order to inactivate the enzyme. A 100 ml sample was centrifuged at ambient temperature for 15 minutes at 3000 x g (g = gravity). The supernatant was ion-exchanged and analysed for carbohydrate composition by HPLC. Also the supernatant was analysed for Kjeldahl—N and dry matter and the nitrogen solubility index (NSI) and the dry matter solubility index (DSI) was calculated; vide results in table Ba I. 100 ml of the reaction mixture cooled to 20°C was poured in a 100 ml graduated glass and kept at 4°C for two days. The dispersion stability (%) was measured by reading the volume of the dispersions obtained (table Ba II) after 1 and 2 days.

To 200 ml of the reaction mixture (at 20°C) was added 8 g of soy bean oil. An emulsion was made by blending for 2 minutes in a Waring Blender. The emulsion stability (%) was measured as above after 1 and 2 days.

B: A reaction was carried out as above, however, in this case 1.00 g of the SPS-ase preparation was used. The same types of analyses and stability measurements as described in section A were performed. Results are shown in tables Ba I and Ba II.

It appears from the chemical analysis of the supernatants that the values for NSI (%) and DSI (%) obtained at the B experiment is higher than for the A experiment. However, the stability tests carried out on the reaction mixtures show a better value for the A samples. This probably is due to the higher peptide chain length of the proteins in the reaction mixture with the low enzyme dosage.

From the carbohydrate composition measured by HPLC it appears that mainly mono- and disaccharides are produced. Thus oligosaccharides known to be responsible for diarrhoea and inflatulence when given to calves in too large amounts are present in small amounts only.

TABLE Ba I

Chemical properties of the supernatant.

Experiments	Enzyme dosage in relation to substrate % w/w	NSI, %* (at pH = 4.5)	DSI, %** (at pH = 4.5)	HPLC results (neutral sugars composition)
A	E/S = 0.5%	39.9	62.4	$DP_1 + DP_2$: 79.7% DP_3 : 7.4% DP_4 : 12.2% DP_{4+} : 8.1%
B	E/S = 2.0%	57.0	67.1	$DP_1 + DP_2$: 84.4% DP_3 : 6.1% DP_4 : 3.7% DP_{4+} : 5.7%

* NSI = Nitrogen Solubility Index
** DSI = Dry matter Solubility Index

TABLE Ba II stability tests of the reaction mixtures

Experiments	Enzyme dosage in relation to substrate % w/w	Stability tests			
		Without oil		With oil	
		Dispersion 1. day	Dispersion 2. day	Emulsion 1. day	Emulsion 2. day
A	E/S = 0.5%	80%	63%	100%	87%
B	E/S = 2.0%	66%	35%	85%	71%

Ba 2. Production of saccharified starch containing raw materials.

In relation to the saccharification of cassava and sweet potatoes and other starch containing plant materials addition of an SPS-ase preparation is capable of solving viscosity problems. By using an SPS-ase preparation it is possible to produce starch suspensions with a dry matter content of 25 to 30%, and after saccharification the mash may be fermented whereby a cheap ethanol may be obtained.

EXAMPLE Ba 2.1

On the basis of fresh and grated sweet potatoes (Japanese) a mash with a dry matter content of 24% was produced. The starch content of sweet potatoes was found to be approximately 70% of the dry matter content thereof. A pre-liquefaction by means of the bacterial amylase of Termamyl (registered Trade Mark) 60 L in a dosage of 0.5 kg/ton of starch was performed by heating the mash to 90°C. The mash was then held at 90°C for 30 minutes. The viscosity τ_1 of the reaction mixture was then measured by means of a HAAKE spindle at 90°C.

Hereafter the reaction mixture was cooled to 55°C, and pH was adjusted to pH 5.0 with 2N H_2SO_4. A saccharification was then initiated by addition of the gluco-amylase SAN 150 (trade mark from NOVO INDUSTRI A/S) in a dosage of 1.75 litre/ton of starch. The saccharification mixture was then divided into three parts, A, B, and C, which were enzyme treated for 15 minutes as shown below before measurement of viscosity:

A: This part is the control. The viscosity τ_2 was measured, vide table Ba III.

B: The Tricoderma viride cellulase of Celluclast (registered Trade Mark) 200 N was added in a dosage of 1 kg/ton of dry matter of sweet potatoes. The viscosity τ_3 was measured, vide table Ba III.

C: The SPS-ase preparation of KRF—68 was added in a dosage of 0.25 kg/ton of dry matter of sweet potatoes. The viscosity τ_4 was measured, vide table Ba III.

TABLE Ba III: Viscosities

Reaction mixture	Viscosity at 90°C	Viscosity at 55°C
Pre-liquefied sweet potatoes	$\tau_1 = 770$ cp	$\tau_2 = 2190$ cp
A	—	$\tau_2 = 2190$ cp
B	—	$\tau_3 = 1970$ cp
C	—	$\tau_4 = 950$ cp

Thus it can be seen that the viscosity of the reaction mixture could be effectively reduced with the SPS-ase in a low dosage compared to the Celluclast (registered Trade Mark) and SAN 150.

Ba 3. Total liquefaction of pears and other fruits.

If whole pears which are mechanically crushed are subsequently treated with an SPS-ase preparation, total liquefaction takes place, and a clear pear juice is produced after removal of minor amounts of solid matter. A similar method can be used in relation to other similar fruits, e.g. apples.

EXAMPLE Ba 3.1

Fresh apples were coarsely milled by means of a Bucher Central mill. The apply mash was then pasteurized in a heat jacketed tank at 90°C for 5 minutes and then cooled to ambient temperature. The pre-mashed apples were then milled on a Fryma mill with corund stone outfit until the mash was smooth to the touch. The mash was then re-pasteurized at 80°C for 10 minutes, and cooled to 50°C.

Enzyme reactions were now carried out at 50°C for 30 minutes with the Contraves Rheomat 15, stirring and viscosity measurement (in relation to a percentage reading on the Rheometer at speed 13) being carried out simultaneously. After completion of the enzyme reactions 100 g of samples were drawn out and centrifuged in a graduated tube at 3000 x g for 15 minutes. Hereby the percentage of juice and the percentage of deposit are measured. pH and the percentage of refractometer dry matter as °Brix were also measured. Table Ba IV shows a comparison between the effect of SPS-ase, the combination of Celluclast and SPS-ase, and the combination of Celluclast an Pectinex. The SPS-ase preparation KRF—68 was used.

TABLE Ba IV

Results of total liquefaction experiments with apple mash at 50°C for 30 minutes.

SPS-ase g/hl mash	Celluclast (registered Trade Mark) 200 l g/hl mash	Pectinex (registered Trade Mark) 3x g/hl mash	Final viscosity %	Centrifugation		Juice	
				% juice	% deposit	pH	°Brix
0	0	0	100	59	41	3.8	9.7
25	0	0	19	81	19	3.5	10.5
50	0	0	15	79	21	3.5	10.7
50	50	0	4.8	83	17	3.5	10.8
			3.8	83	17	3.1	12.5
0	50	200	9.5	83	17	3.2	12.9
0	50	2000	4.0	82	18	3.1	13.2

Ba 4. Production of juice by treatment of fruits and vegetables.

It has been found that SPS-ase preparations are well suited for production of juice by treatment of several fruits, berries and vegetables, e.g. carrots, peas, tomatoes, apples, pears, black currents, beans and cabbage. Hereby an improved juice yield, and better extraction of colour and flavour components are achieved compared to commercially available pectinase and cellulase preparations.

EXAMPLE Ba 4.1

Reference is made to example Ba 3.1 where the SPS-ase preparation has been compared to the commercially available conventional cellulase and pectinase products Celluclast (registered Trade Mark) 200 L and Pectinex (registered Trade Mark) 3x. It appears from the table that the juice yield may be slightly improved with as little as 50 g/hl of SPS-ase compared to 2000 g/hl of Pectinex (registered Trade Mark), both in combination with 50 g/hl of Celluclast (registered Trade Mark). Also the viscosity was slightly lower. Thus it seems that the SPS-ase is about 40 times more effective than Pectinex.

Ba 5. Treatment in relation to extraction or pressing of sugar cane or sugar beet.

It has been found that it is possible to improve the yield related to simple extraction processes, if the SPS-ase preparation is used for treatment of sugar cane or sugar beet before and/or during the extraction or pressing thereof. Also, the remanence (the bagasse) can be treated with the SPS-ase preparation, whereby it is partially converted to fermentable sugars which may be used as a raw material for ethanol fermentation.

EXAMPLE Ba 5.1

10 kg of sugar beet remanence (pulp) obtained from continuous countercurrent extraction in a DDS-diffuser at Nakskov Sugar Factory was milled in a Fryma mill (type MZ—110). Processing water was added during the milling operation.

300 g portions of the pulp were now enzyme treated at 45°C for 18 hours by means of the enzyme dosages shown in table Ba V. The dry enzyme product (KRF—68) was added to the pulp which was stirred by a rod during the first hour. Thereafter the pulp was liquefield to such an extent that magnet stirring could successively be performed for the remaining time. At the end of the reaction pH was measured (no pH-corrections were made during the start of the reaction) and the reaction mixture was centrifuged until a clear supernatant was obtained. Dry matter determinations were performed on the reaction mixtures and on the supernatants. Based on these results the percentages of solubilized dry matter were calculated. Corrections for the soluble dry matter of the enzyme product were made in all calculations.

Supernatants Nos. 2, 3, and 4 were ionexchanged and analysed by HPLC for carbohydrate composition.

TABLE Ba V

Results obtained by enzymatically liquefaction of beet pulp.

Experiment No.	Enzyme dosage in relation to to dry matter, E/S%	Final measurements			
		Reaction mixture		supernatents	
		pH (final)	% dry matter	% dry matter	% solubilized dry matter
1	0	5.5	4.18	0.0	0.0
2	0.35	3.6	3.85	2.58	66.9
3	0.56	3.5	3.81	2.56	66.2
4	1.02	3.5	3.86	2.73	70.4
5	1.58	3.3	3.17	2.34	73.4
6	3.10	3.4	3.23	2.49	76.4
7	7.52	3.4	2.66	2.18	80.5

Reaction conditions: M = 300 g
S = 4.18% dry matter
E/S as shown above
pH not adjusted
T = 45°C
t = 18 hours

TABLE Ba VI

HPLC data.

Sugar type (Neutral)	Experiment No.		
	2	3	4
	% of neutral sugars		
High molecular (DP4+)	43.6	31.9	25.3
Disaccharides	4.6	4.8	—
Glucose	20.4	23.7	27.8
Galactose	5.0	5.9	7.3
Fructose/Arabinose	26.4	32.2	33.2
Galacturonic acid	not measured		

All the sugars formed according to the above table Ba VI could be fermented to alcohol or used for other purposes.

Ba 6. Production of soy milk.

Soy milk can easily be produced by total liquefaction of milled soy beans and subsequent homogenization of the resulting mixture. Soy milk is often produced by soaking of soy beans in boiling water, milling of the soaked beans and water extraction followed by a separation of insoluble residues, e.g. proteins and polysaccharides. In order to improve the yield of the soy milk these insoluble residues may be liquefield by reaction with the SPS-ase.

EXAMPLE Ba 6.1

The soy milk process is illustrated by the following series of enzyme reactions, whereby calculations of the protein solubility index (PSI, %) and the dry matter solubility index (DSI, %) illustrate the yields obtained after separation at pH = 7 (vide table Ba VII). The enzyme reactions were carried out under the following conditions:

Substrate: Full fat soy flour (Dansk Sojakagefabrik A/S)
Mass of reaction mixture: 220 g
Mass of substrate: 20 g
Temperature: 50°C
pH: 4.5 (6 N HCl)
Reaction time: Series A: 1 hour
— — Series B: 0.5—6 hours
Enzyme: SPS-ase (KRF—68)
Enzyme dosage: Series A: E/S-ratio (w/w): 0—8.0%
Series B: E/S-ratio (w/w): 1.0%

After the reaction pH was adjusted to pH = 7 by means of 4 N NaOH, and a separation was performed by centrifugation at 3000 x g for 15 minutes.

TABLE Ba VII. Mass balance calculations in relation to the enzymatic soy milk process.

Series	Reaction time hours	Enzyme-dosage E/S %	Reaction mixture		Supernatant		Solubility indices	
			% protein	% dry matter	% protein	% dry matter	PSI %	DSI %
A	1.0	0	3.65	8.70	1.87	5.72	49.6	63.7
	1.0	0.5	3.68	8.74	2.41	6.28	63.7	70.0
	1.0	1.0	3.71	8.78	2.48	6.43	65.1	71.4
	1.0	2.0	3.78	8.86	2.98	7.18	77.5	79.5
	1.0	4.0	3.92	9.03	3.36	7.69	84.6	83.9
	1.0	8.0	4.19	9.37	3.76	8.08	88.5	85.0
B	0.5	1.0	3.64	8.78	2.39	6.41	64.0	71.1
	1.0	1.0	3.64	8.78	2.56	6.60	68.7	73.4
	2.0	1.0	3.63	8.78	2.79	6.91	75.2	77.1
	4.0	1.0	3.63	8.78	3.10	7.29	84.0	81.7
	6.0	1.0	3.63	8.78	3.39	7.69	92.3	86.5

Ba 7. Treatment in order to increase the recoverable amount of coffee solubles.

It has been found that treatment of coffee beans at different stages during the production of instant coffee results in an increased yield of coffee solubles. Thus, e.g. spent coffee grounds or green beans can be enzyme treated with favourable results.

Bb 1. Prevention and/or decomposition of apple or pear haze.

After production of apple juice or pear juice and other fruit juices, which have to be clear, and which are previously treated with conventional pectinase and cellulase preparations in order to prevent formation of turbidity, an apple haze or similar fruit hazes may appear. It has been found that the SPS-ase preparations are well suited for decomposition of such hazes, which mainly consist of araban bound to proteins.

EXAMPLE Bb 1.

Pear juice concentrate produced by enzymatic liquefaction of pear cannery waste during Celluclast (registered Trade Mark) and Pectinex (registered Trade Mark) was found to be cloudy on standing. The haze was isolated and hydrolyzed with 0.01 N H_2SO_4 for 24 hours and analyzed by HPLC. The chromatogram showed arabinose and small amounts of aligosaccharides.

By incubation of 0.5% w/v of this carbohydrate in 1 mM acetate buffer at pH 4.5 for 3 hours at 40°C with SPS-ase (KRF—68 + KRF—92 1:1) with an enzyme concentration of 0.05% w/v it was found that 84% of the initial haze carbohydrate (dry matter) was converted to arabinose.

Also diluted pear concentrate (20°Brix) was treated for 2 hours at 40°C with an enzyme dosage of

the above mentioned SPS-ase of 0.15% w/v or with a commercial product called Clarex (registered Trade Mark) in a dosage of 1% w/v. It was found that the SPS-ase was able to reduce the relative HPLC peak area of an araban-like haze with 86%, whereas the corresponding reduction with Clarex (registered Trade Mark) (used in a much higher dosage than the SPS-ase preparation) was only 78%.

Bb 2. Use as a clarifying agent for white wine.

It has been found that white wines exhibiting a highly undesired turbidity can be effectively clarified by means of SPS-ase. It has been shown that the cloudy material mainly consists of arabinogalactans which are bound to hydroxyprolin residues in a cell wall structural protein.

Bb 3. Production of ISSPH or other vegetable protein hydrolyzates.

Previously to the separation of the ISSPH (isoelectric soluble soy protein hydrolyzate) or other vegetable protein hydrolyzates from the sludge, as described in US patent No. 4,100,024 or in Process Biochemistry, vol. 14, No. 7 (1979), pages 6 to 8 and 10 to 11, the reaction mixture can be treated with an SPS-ase preparation. Hereby an easier separation is obtained.

Bb 4. Mashing enzyme in the brewing industry.

In production of beer carbohydrates of the raw materials, e.g. the beta-glucans of malt and barley, influence the viscosity and filterability of the wort. Addition of SPS-ase during mashing will reduce wort viscosity and improve filterability and extract yield. Furthermore addition of SPS-ase during mashing will increase the fermentability of the wort and the nitrogen content in the wort.

EXAMPLE Bb 4.1

In the laboratory 50 g of milled grits consisting of 50% malt and 50% barley were mashed together with 275 g of water (15% dry matter) according to the following mashing diagram: 52°C (60 minutes)/63°C (60 minutes)/76°C (30 minutes).

In order to demonstrate the effect of the SPS-ase four tests were carried out, vide the below indicated table, whereby the enzymes were added during mashing in (pH of mash 5.5—6.0).

Enzyme	None	Cereflo	SPS-ase (KRF 68)	
Activity of beta-glucanase/g	0	200 BGU	1630 BGU	1630 FBG
Dosage of enzyme per kg grits	0	1.5 g	0.05 g	0.18 g
Total dosage of enzyme activity units per kg grits	0	300 BGU	80 FBG	300 FBG
Filtration rate of wort after 10 minutes	120 ml	135 ml	160 ml	170 ml
Viscosity of wort 10° Balling (25°C)	1.52 cP	1.36 cP	1.36 cP	1.30 cP

BGU is beta-glucanase units determined according to analytical method AF 70/4—GB obtainable from NOVO Industri A/S.

FBG is fungal beta-glucanase units determined according to analytical method AF 70.1/2—GB obtainable from NOVO Industri A/S.

The only difference between BGU and FBG is the pH, at which the enzyme determination is carried out: pH 7.5 for BGU and pH 5.0 for FBG.

Cereflo is a bacterial beta-glucanase preparation described in the information leaflet B 214b—GB 1500 July 1981 available from NOVO Industri A/S.

EXAMPLE Bb 4.2

In the laboratory 50 g of milled grits consisting of 40% malt and 60% barley were mashed together with 150 g of water (25% dry matter) according to the following mashing diagram: 45°C (60 minutes)/63°C (90 minutes)/75°C (15 minutes).

In order to demonstrate the effect of the SPS-ase three tests were carried out, vide the below indicated table, whereby the enzymes were added during mashing in (pH of mash 5.5—6.0).

Enzyme	None	Ceremix	SPS-ase (KRF 68) + Ceremix added as in previous test
Activity of beta-glucanase/g	—	200 BGU	1630 FBG
Dosage of enzyme per kg grits	—	1.65 g	0.033 g
Total dosage of enzyme activity units per kg grits	0	330 BGU	50 FBG
Filtration rate of wort after 30 minutes	48 ml	98 ml	111 ml
Extract, °Balling	18.6	19.0	19.5
Viscosity of wort 10° Balling (25°C)	1.72 cP	1.37 cP	1.27 cP

The definition of BGU and FBG is as indicated in Example Bb 4.1. In the last column in the above table only the activity and dosage originating from SPS-ase is indicated.

Ceremix is a bacterial beta-glucanase preparation described in the information leaflet B 216 b—GB 1000 Feb. 1982 available from NOVO Industri A/S.

Bb 5. Enzymatic additive for use during beer fermentation and/or storage.

SPS-ase can be added during fermentation of wort or storage of beer in order to reduce the content of beta-glucans and thereby improve beer filtration and beer stability in regard to haze. SPS-ase will also exert an effect on proteins responsible for chill haze.

Bb 6. Almond skin release agent.

During the mechanical almond skin removal step following the blanching of almonds a certain percentage of the almond skins are not released. It has been found that enzyme treatment of the almonds results in a decrease of the above indicated percentage.

Bc 1. Decomposition of different waste materials.

In relation to certain manufacturing processes large amounts of carbohydrate containing waste materials are formed. For instance this is the case in relation to production of soy isolate by water extraction and acid precipitation, soy milk and tofu (a special kind of Japanese cheese). Also in this connection waste pulp from e.g. apples, pears, or citrus fruits may be mentioned. It has been found that an SPS-ase preparation is able to liquefy this carbohydrate containing waste material completely and to produce fermentable sugars which can be used as a starting material for ethanol fermentation.

EXAMPLE Bc 1.1

By traditional production of soy milk or tofu soy beans are often soaked in boiling water, milled and extracted with hot water, whereafter a separation is carried out. The residue from this separation is the material used for this experiment. The liquid phase is the soy milk, which may be further treated to produce tofu.

10 kg of whole soy beans obtained from Aarhus Oliefabrik A/S was milled simultaneously with 70 litres of boiling water in a Fryma mill type MZ 110. The milled slurry was then held above 85°C for 15 minutes in order to inactivate the natural bean enzymes which develop the well-known soy bean off-flavour. 5 litres of this soy bean slurry was then centrifuged in the laboratory for 15 minutes at 3000 x g (g = gravity). It was found by analysis that the remanence contained 20.45% and 20.06% dry matter (duplicate determinations, calculated average 20.26%). 6 N HCl was slowly added and worked into the remanence with a spatula until a pH-meter showed 4.50, when the electrode was introduced directly into the mass.

Enzyme reactions on 2 x 200 g of the mass with the two dosages of the SPS-ase (KRF—68) E/S = 0.5% in relation to dry matter and E/S = 3.0% in relation to dry matter were carried out in a 500 ml beaker at 50°C. The dry enzyme was added to the mass. During the first 1 to 2 hours the stirring was carried out with a spatula and hereafter the mass was liquified to such an extent that stirring with a magnet could be carried out successfully. The total reaction time was 21 hours. During the reaction the osmolality was measured with an osmometer (Advanced Digimatic 3DII from Advanced Instruments

Inc.). The results in table Bc I show the course of the reaction. At the end of the experiment the mixtures were centrifuged at 3000 x g for 15 minutes. A layer of oil appeared on the top of the supernatants and the volume thereof was determined. A loose layer of sludge appeared as a bottom-layer. The supernatant including the oil was removed with a pipette. The oil was combined with the clear water phase by homogenisation and a sample was drawn for dry matter determinations. The results shown in table Bc I clearly demonstrate that this waste product can be liquefied by an enzymatic reaction and that crude oil can be produced as mentioned in section A4. After recovery of the oil the solubilized remanence may be used in different ways, e.g. for fermentation to valuable compounds or for concentration and drying and subsequent use as a fodder or food product, or after further purification for production of valuable products.

TABLE Bc I

Results obtained during liquefaction of soy milk and tofu-sludge.

Reaction conditions and results	Experiment A			Experiment B		
Mass of residue	200 g			200 g		
Mass of SPS-ase (KRF-68)	0.20 g			1.20 g		
Temperature	50°C			50°C		
pH	4.50			4.50		
Reaction time	21 hours			21 hours		
Results measured on osmometer during the course of the reaction	t min.	osmolality mOsm	Δosmolality mOsm	t min.	osmolality mOsm	Δosmolality mOsm
Δ Osmolality is the value	0	287	0	0	282	0
corrected for the osmolality of	10	313	26	10	368	86
the mixture at t = 0	—	—	—	25	497	215
	40	391	104	45	601	319
	95	501	214	95	718	436
	250	634	347	250	875	593
	1260	907	620	1260	1145	863
Reaction mixture: Dry matter	20.3%			20.7%		
Supernatant: Dry matter	18.0%			19.4%		
Supernatant: Oil content	8—10%			8—10%		
Calculation % solubilized dry matter	88.6%			93.5%		

Bc 2. Saccharification and simultaneous fermentation.

Carbohydrate containing plant materials, e.g. tubers like Jerusalem artichokes, potatoes, sweet potatoes, cassawa, or pulp from such tubers, i.e. the material remaining after removal of the extracted components may be saccharified by treatment with an SPS-ase preparation and simultaneously the formed fermentable saccharides may be fermented to ethanol.

EXAMPLE Bc 2.1

The production of ethanol by fermentation of the decomposed inulin containing Jerusalem artichokes was examined in laboratory scale by simultaneous saccharification with SPS-ase and inulinase and by four different pretreatments of the Jerusalem artichokes.

SPS-ase: The SPS-ase preparation KRF—68 was used.
Inulinase: The inulinase was produced by fermentation of Asp. ficuum (CBS 55 565). The inulinase activity is determined as described in Research Disclosure No. 21234 (December 1981) p. 456 to 458.
Laboratory fermentation: 150 g portions of the pretreated mash (described later) were fermented after addition of 4.5 g of bakers yeast and 1 ml of a 4% solution of Pluronic as an antifoaming agent. The fermentation flasks are provided with CO_2 traps containing 98% sulphuric acid, and the fermentation is followed by measurement of the weight loss due to liberated CO_2. The content of the flasks is agitated during the fermentation carried out at 30°C. Three flasks were used for each parameter studied.

In table Bc II the weight loss due to liberation of CO_2 is converted to ethanol assuming that 1 mol liberated CO_2 is equivalent to 1 mol C_2H_5OH,

$$\text{i.e. } 1 \text{ g } CO_2 \sim \frac{46}{44} \text{ g } C_2H_5OH.$$

Pretreatments of the artichokes:
Treatment A: 14.1 kg artichokes (22.8% dry matter) was Henze-cooked at 140°C and 4 to 5 atm. for 20 minutes. The weight after cooking was 19.0 kg (~16.9% dry matter). Fermentations were performed directly on the mash.
Treatment B: Washed and sliced artichokes were mixed with water (1:1) and blended in a Waring blender. The mash was then heat treated for one hour at 85°C and pH = 4.5.
Treatment C: As B, but pH was not adjusted.
Treatment D: As B, but no heat treatment and no pH adjustment.

Results: In table Bc II the results show the effect of addition of SPS-ase to the pretreated mash on the ethanol yield. A significant improvement of the ethanol yield was obtained on all pretreated mashed when SPS-ase was added.

TABLE Bc II

Fermentation results in relation to simultaneous fermentation and enzyme saccharification of Jerusalem artichokes.

Pre-treatment	Inulinase units added to 1 g of dry matter	SPS-ase E/S %	Loss of CO_2 (g) after 42 to 44 hours of fermentation	% ethanol produced in relation to dry matter
A	1.5	0	7.65 ± 0.05	31.5
	1.5	0.27	8.07 ± 0.08	33.2
B	1.5	0	4.85 ± 0.03	29.7
	1.5	0.40	5.41 ± 0.03	33.1
C	1.5	0	5.70 ± 0.05	34.8
	1.5	0.10	5.97 ± 0.01	36.5
	1.5	0.20	6.13 ± 0.06	37.5
	1.5	0.30	6.13 ± 0.00	37.5
	1.5	0.40	6.18 ± 0.05	37.8
D	1.5	0	5.77 ± 0.02	35.3
	1.5	0.10	5.89 ± 0.00	36.0
	1.5	0.20	6.04 ± 0.11	36.9
	1.5	0.30	6.01 ± 0.01	36.7
	1.5	0.40	6.02 ± 0.03	36.8
	0	0.40	5.48 ± 0.02	33.5

Bc 3. Decomposition of cellulose.

It has been found that cellulose containing materials like straw, e.g. wheat straw, saw dust, paper, and lignocellulose, may be hydrolyzed to a greater extent with an SPS-ase preparation than with conventional cellulases. This is illustrated by the following example in which a crystalline cellulose material (AVICEL) is treated by means of a conventional cellulase Celluclast (registered Trade Mark) 200 produced by Trichoderma reesei and the SPS-ase preparation KRF—68.

EXAMPLE Bc 3.1

Avicel was suspended in water (20% dry matter); pH was adjusted to 5, and the temperature was maintained at 50°C. After 24 hours reaction time the slurry was filtered, and the content of reducing sugar (mg glucose/g AVICEL) was measured. Using enzyme dosages of 5% and 20% of the cellulose content the following values were found:

TABLE Bc III

Enzyme	E/S %	mg glucose/ g AVICEL
Celluclast	5	80
SPS-ase	5	200
Celluclast	20	100
SPS-ase	20	340

Bc 4. Application as a baking aid.

It has been found that SPS-ase preparations are excellently suited as baking aids. Thus, when an SPS-ase preparation is added to the dry flour, before production of the dough, it is possible to obtain a bread with superior quality in regard to volume, crumb and taste. Thus it is possible to obtain a high quality bread with a low quality wheat flour if an SPS-ase preparation is used as an additive.

Bc 5. Improvement of alcohol yield and yield of biomass during fermentation of sulphite liquor from paper production.

Also it is found that the yield of ethanol may be improved if paper sulphite liquor is treated with an SPS-ase preparation before it is utilized as a carbohydrate source for fermentation of ethanol. Paper sulphite liquor may also be used for production of biomass, e.g. single cell protein, by fermentation, and also in this case the yield of biomass has been improved when the sulphite liquor has been treated with an SPS-ase preparation previously. Also, decomposition due to the presence of the SPS-ase preparation and fermentation can be performed simultaneously.

Bc 6. Dewatering of biological sludge products.

During traditional water extraction of many biological materials from plant raw materials, large volumes of insoluble residue consisting of great proportions of swelled polysaccharides are formed. This, for example, is the case when soy milk, tofu, or soy isolate is produced by water extraction of soy beans, defatted soy flour, or white flakes. The structural swelled polysaccharide material may then be treated to a slight extent with SPS-ase, whereby the network structure of the material is opened, and only slight amounts of carbohydrates are solubilized. Thereby the material is dewatered, and consequently a higher dry matter content is obtained in the sludge in comparison to a product obtained without the enzymatic treatment. Thus, the enzyme process exhibits the advantage of a considerably lower energy consumption for removal of water by drying, and it also opens up the possibility for production of a cheaper dry animal feed material or bulking agent for food applications.

Bc 7. Silage aid.

It is known to add enzymatic silage aids to silage in order to increase the rate of the silage process and the digestability of the silage. It has been found that SPS-ase preparations are superior in comparison to known enzymatic silage aids.

A survey of the figures, to which reference has been made already, is given below for the purpose of providing a better comprehensive view.

Fig. No.	Belongs to	Describes
1	The general part of the specification	Demonstration of binding effect between SPS and soy protein
2	The general part of the specification	Flow sheet describing the production of SPS
3	Section 2	Calibration curve for HPLC gel filtration chromatography
4	Section 2	HPLC gel filtration chromatogram of SPS
5	Section 2	HPLC gel filtration chromatogram of SPS decomposed by SPS-ase
6	Section 2 and 3	HPLC gel filtration chromatogram of supernatant from SPS incubated with soy protein
7	Section 2	HPLC gel filtration chromatogram of supernatant from decomposed SPS incubated with soy protein
8	Section 3	HPLC gel filtration chromatogram of APS decomposed by Pectolyse
9	Section 3	HPLC gel filtration chromatogram of APS decomposed by SPS-ase
10	Section 3	HPLC gel filtration chromatogram of SPS treated with Pectolyase
11	Section 7	Immunoelectrophoretic peaks including an SPS-ase peak identified by overlay technique
12	Section 8	Ion exchange chromatogram of an SPS-ase
13	Section 9	pH-activity dependency of an SPS-ase
14	Section 9	Temperature activity dependency of an SPS-ase
15	Section 9	Temperature stability of an SPS-ase
16	Section 10	pH-stability of protease in an SPS-ase preparation

CLAIMS

1. An SPS-ase, a carbohydrase in a usable form and capable of decomposing soy SPS under appropriate conditions into decomposition products which attach themselves to protein in an aqueous medium to a lesser extent than the soy SPS prior to decomposition would have attached itself to the same protein under corresponding conditions.

2. An SPS-ase according to claim 1 wherein the SPS-ase is capable of decomposing soy SPS in an aqueous medium into decomposition products which attach themselves to vegetable protein in the aqueous medium to a lesser extent than the soy SPS prior to decomposition would have attached itself to the same vegetable protein in the aqueous medium.

3. An SPS-ase according to claim 1 or 2, wherein the SPS-ase is capable of decomposing soy SPS in an aqueous medium with a pH value not deviating more than 1.5 from 4.5 into decomposition products which attach themselves to soy protein in the aqueous medium to a lesser extent than the soy SPS prior to decomposition would have attached itself to the soy protein in the aqueous medium.

4. An SPS-ase according to claim 1 to 3, wherein the decomposition products of soy SPS after completed degradation attach themselves to the vegetable protein to an extent of less than 50% particularly less than 20% than the soy SPS prior to decomposition would have attached itself to the vegetable protein in the aqueous medium.

5. An SPS-ase according to claim 1 to 4, wherein the SPS-ase exhibits a positive SPS-ase test, when examined according to the qualitative and quantitative SPS-ase determination method.

6. An SPS-ase according to claim 1 to 5, wherein the SPS-ase was produced by means of a microorganism belonging to the genus Aspergillus, preferably belonging to the Aspergillus niger group.

7. An SPS-ase according to claim 1 to 6, wherein the SPS-ase is derived from the enzymes producible by means of Asp. aculeatus CBS 101.43.

8. An SPS-ase according to claim 1 to 7, wherein the SPS-ase is immunoelectrophoretically identical to the SPS-ase producible by means of Asp. aculeatus CBS 101.43 and identifiable by means of the immunoelectrophoretic overlay technique.

9. Isolated SPS, wherein the isolated SPS is produced on the basis of vegetable raw protein as a raw material.

10. Isolated SPS according to claim 8, wherein the vegetable raw protein is defatted soy meal.

11. Method for selection of an SPS-ase producing microorganism, for production of the SPS-ase according to claim 1 to 8, wherein the microorganism to be tested is grown on a fermentation medium, the main carbon source of which is SPS according to claim 9 or 10, whereafter a sample of the fermentation medium is analyzed for SPS-ase and the microorganism in question is selected as an SPS-ase producing microorganism, if the analysis for SPS-ase is positive.

12. Method for production of SPS-ase according to claim 1 to 8, wherein a strain selectable according to the method of selection according to claim 11 is cultivated in a nutrient medium.

13. Method according to claim 12, wherein the strain Asp. aculeatus CBS 101.43 or Asp. japonicus IFO 4408 is cultivated in a nutrient medium.

14. Method according to claim 12 or 13, wherein the cultivation is carried out as a submerged cultivation at a pH in the range of from 3 to 7, preferably from 4 to 6, at a temperature in the range of from 20 to 40°C, preferable from 25 to 35°C, and whereby the nutrient medium contains carbon and nitrogen sources and inorganic salts.

15. Method according to claim 12 to 14, wherein the nutrient medium contains soy meal.

16. Method according to claim 15, wherein the soy meal is treated with a proteolytic enzyme before the use as a component of the substrate, preferably the proteolytic enzyme produced microbially by means of Bacillus licheniformis.

17. Method according to claim 12 to 16, wherein a sterile solution of pectin is added aseptically to the fermentation broth during the cultivation.

18. Method for decomposition of polysaccharides, preferably plant cell wall polysaccharides, by means of a carbohydrase, wherein as SPS-ase preparation according to claim 1 to 8 in an aqueous medium is contacted with a substrate for said SPS-ase preparation.

19. Method for decomposition of polysaccharides according to claim 18, wherein the decomposition is accompanied by the isolation or extraction of a biological material other than soy protein and related vegetable proteins from a raw biological material, whereby the SPS-ase preparation is essentially free of any enzyme which is able to degrade said biological material.

20. Method for decomposition of polysaccharides according to claims 18 or 19, wherein one or more of the reaction products (no matter whether they are wanted and products or waste products) are treated further simultaneously with or after the enzyme treatment.

21. Method for decomposition of polysaccharides according to claim 20, wherein the further treatment is an alcoholic fermentation in case one of the reaction products is a fermentable sugar.

22. Any novel feature or combination of features described herein.

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